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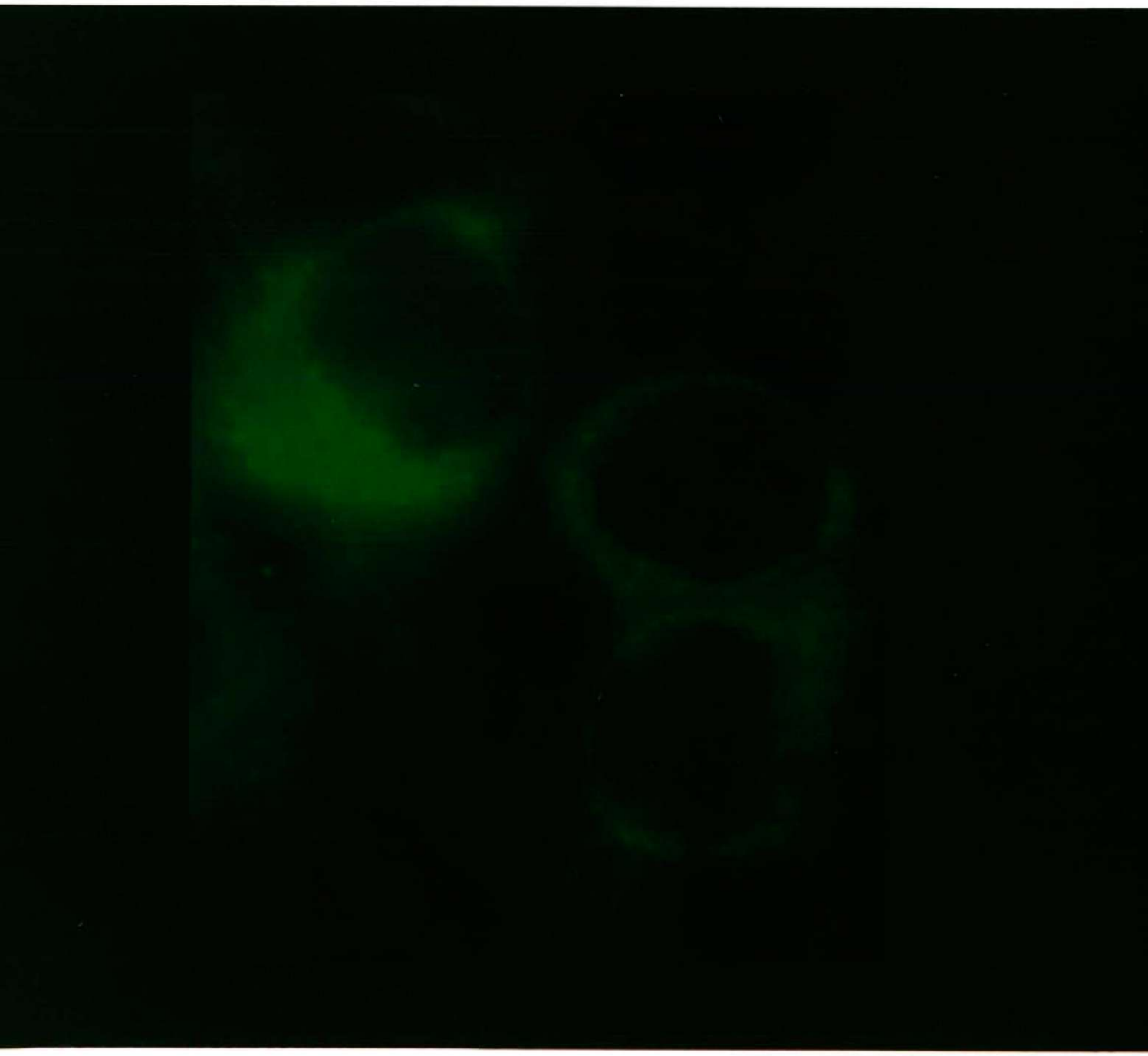

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RIVEW ARTICLE

Serotonin receptors and systems: endless diversity?

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ABSTRACT Serotonin is probably unique among the monoamines in that its effects are subserved by as many as 13 distinct heptahelical, G protein-coupled receptors (GPCRs) and a ligand-gated ion channel family (5-HT_3). These receptors are divided into seven distinct classes (5-HT_1 to 5-HT_7) largely on the basis of their structural, transductional and operational characteristics. While this degree of physical diversity clearly underscores the physiological importance of serotonin, evidence for an even greater degree of operational diversity continues to emerge. Here, we will review this diversity and its physiological and possibly pathophysiological consequences. Indeed, 5-HT research which is about 50 years old, has resulted in numerous therapeutic agents, some of which have a major impact on disease management. Thus, selective 5-HT reuptake inhibitors (SSRIs) are among the most widely used drugs in depression and other disorders such as anxiety, social phobia, panic disorders, or obsessive compulsive disorders (OCDs) to name a few. The discovery of 5-$HT_{1B/1D}$ receptor agonists (the triptans) for treating migraine, 5-HT_3 receptor antagonists for chemotherapy and radiation-induced emesis, and finally the emergence of 5-HT_3/ 5-HT_4 ligands to treat irritable bowel syndrome (IBS), all represent major advances in the field. Finally, the role of 5-HT in the mechanism of action of antipsychotic agents still is a topic of intense research, which promises better treatments for schizophrenia.
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KEY WORDS

serotonin
5-HT
5-hydroxytryptamine
receptor families and subtypes

Serotonin (5-hydroxytryptamine; 5-HT) produces its effects through a variety of membrane-bound receptors both in the central and peripheral nervous system (CNS/PNS) as well as in a number of non-neuronal tissues (e.g. gut, cardiovascular system and blood). The main source of 5-HT is in the gut, more precisely enterochromaffin cells, where it is synthesised from tryptophan. It can be released into the gut lumen e.g. as a reaction to pressure and act on receptors located on the smooth muscle, or into the portal blood circulation, by a variety of nervous or alimentary stimuli. 5-HT is also found in enteric neurones. In the blood, the vast majority of 5-HT is not free, but to be found in the platelets, which are endowed with a very active uptake system (they do probably not synthesise 5-HT) and 5-HT is stored in storage granules. Large amounts of 5-HT are released during platelet aggregation, and it can act locally on endothelial cells and vascular smooth muscle. 5-HT is also found in mast cells. In the central and peripheral nervous system, 5-HT acts as a neurotransmitter on a large variety of receptors, which may be located pre or post synaptically. 5-HT is also found in the pineal gland, where it is believed to serve essentially as a precursor for the synthesis of melatonin by 5-HT-N-acetyltransferase and hydroxyindole-O-methyltransferase, under the control of the clock in the suprachiasmatic nucleus which during the circadian rhythm modulates enzyme activity levels up to 50 fold.

The serotoninergic system is one of the oldest neurotransmitter/hormone systems in evolution, which may explain why 5-HT interacts with such a diversity of receptors of the G protein coupled family and the ligand gated family, similarly to acetylcholine, GABA or glutamate. 5-HT was discovered in the gut in the 1930s and called enteramine, then rediscovered in the 1940s in the blood and called serotonin, as it had vasoconstrictor features. 5-HT is synthesised from L-tryptophan, the tryptophan hydroxylase forming 5-hydroxytrytophan (5-HTP), which by the L amino acid decarboxylase leads to 5-HT; serotonin can be conjugated with glucuronide or sulfate or in nerves metabolised via monoamine oxydase to 5-hydroxyindolacetaldehyde and finally to 5-hydroxyindolacetic acid (via aldehydedehydrogenase). It can also lead to 5-hydroxytryptophol by an aldehyde-reductase in some peripheral nerves. Thus, 5-HT acts both as a neurotransmitter with all the features, such as intracellular storage, activity dependent release, the existence of both pre- and postsynaptic receptors, an active uptake system, via the serotonin transporter and metabolising/inactivating enzymes and a hormone, released into the blood or gut to work more distantly.

With the exception of 5-HT_3 receptors (ligand-gated ion channels), 5-HT receptors belong to the G protein-coupled receptor (GPCR) superfamily and, with at least fourteen distinct members, represents one of the most complex

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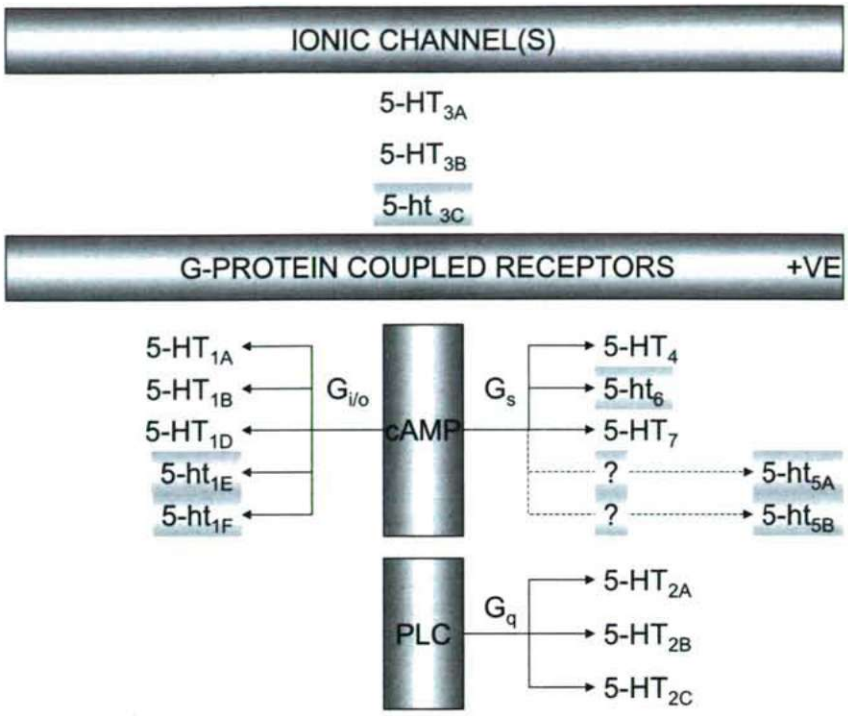

Receptor subtypes represented by shaded boxes and lower case designate receptors that have not been demonstrated to definitively function in native systems. Abbreviations: 3'-5' cyclic adenosine monophosphate (cAMP); phospholipase C (PLC); negative (-ve); positive (+ve).

Figure 1. Graphical representation of the current classification of 5-hydroxytryptamine (5-HT) receptors.

families of neurotransmitter receptors. Multiple splice variants (5-HT_4, 5-HT_7) or RNA edited isoforms (5-HT_{2C}) have been described; there is also evidence that homo- and heterodimerisation (5-$HT_{1B/1D}$) can occur. Furthermore, peptide or lipid modulators of 5-HT receptors have been described such as 5-HT moduline (Leu-Ser-Ala-Leu (LSAL), a putative product of a chromogranin), which has selectivity for the 5-HT_{1B} and 5-HT_{1D} receptors, or oleamide, which acts on several receptors (e.g. 5-$HT_{2A/2C}$ and 5-HT_7).

The 5-HT receptor family has been a target of intense research, in both academia and the pharmaceutical industry, with the identification of more potent and selective ligands for the different receptor subtypes as a major goal. Such selective receptor probes should help to better define the function(s) of these receptors, and lead to drug treatments with fewer side effects. Molecular genetics offer another approach for studying distinct 5-HT receptor subtypes via the generation of gene-targeted and transgenic lines of mice with altered expression of 5-HT receptor or transporter genes. 5-HT is also a substrate for the 5-HT transporter, itself an important target in the treatment of depression and social phobia. It is the target for selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, paroxetine and citalopram. Thus, 5-HT has been implicated in the aetiology of numerous disease states, including depression, anxiety, social phobia, schizophrenia, obsessive-compulsive, panic-disorders, migraine, hypertension, pulmonary hypertension, eating disorders, vomiting and irritable bowel syndrome (IBS).

Current criteria for classifying 5-HT receptors

The classification of 5-HT receptors began in 1957, when it was found that 5-HT effects in the guinea pig ileum could be blocked in part by morphine (M), and in part by dibenzyline (D). Gaddum and Picarelli proposed a subdivision into 5-HT M and 5-HT D receptors (Gaddum and Picarelli 1957). However, neither morphine nor dibenzyline are selective. In 1976 utilising radioligand-binding, the presence of 5-HT receptors was postulated in brain. Then in 1979, Peroutka and Snyder demonstrated the presence of two distinct 5-HT receptor binding sites, using [^{3}H]5-HT, [^{3}H]spiperone, and [^{3}H]LSD. These sites were named 5-HT_1 and 5-HT_2. The M receptor was distinct from the 5-HT_1 and 5-HT_2 receptors in both function and distribution, whereas the D receptor corresponded pharmacologically to the 5-HT_2 binding site. As a result, Bradley and colleagues (1986) proposed the existence of three groups of 5-HT receptors, named 5-HT_1-like, 5-HT_2 and 5-HT_3, the latter corresponding to the M

Table 1. 5-HT_1 receptor nomenclature proposed by the NC-IUPHAR Subcommittee on 5-HT receptors.

Nomenclature	5-HT_{1A}	†§5-HT_{1B}	†5-HT_{1D}	5-ht_{1E}	5-ht_{1F}
Previous names	-	5-$HT_{1D\beta}$	5-$HT_{1D\alpha}$	-	5-$ht_{1E\beta}$, 5-HT_6
Selective agonists	8-OH-DPAT	Sumatriptan L 694247	Sumatriptan PNU 109291	-	LY 334370
Selective antagonists (pK_B)	(±)WAY 100635 (8.7)	GR 55562 (7.4) SB 224289 (8.5) SB 236057 (8.9)	BRL 15572 (7.9)	-	-
Radioligands	[^{3}H]WAY100635 [^{3}H]8-OH-DPAT	[^{125}I]GTI [^{125}I]CYP (rodent) [^{3}H]Sumatriptan [^{3}H]GR 125743	[^{125}I]GTI [^{3}H]Sumatriptan [^{3}H]GR 125743	[^{3}H]5-HT	[^{125}I]LSD [^{3}H]LY 334370
G protein effector	$G_{i/o}$	$G_{i/o}$	$G_{i/o}$	$G_{i/o}$	$G_{i/o}$
Gene/Chromosomal localisation	*HTR1A*/5q11.2-q13	*HTR1B*/6q13	*HTR1D*/1p34.3-36.3	*HTR1E*/6q14-15	*HTR1F*/3p11-p14.1
Structural information	h421 P8908 m421 Q64264 r422 P19327	h390 P28222 m386 P28334 r386 P28564	h377 P28221 m374 Q61224 r374 P28565	h365 P28566	h366 P30939 m366 Q02284 r366 P30940

†5-HT_{1B} and 5-HT_{1D} receptor nomenclature has been revised (Hartig et al. 1996); only the non-rodent form of the receptor was previously called 5-$HT_{1D\beta}$. §Displays a different pharmacology to the rodent form of the receptor.

receptor. The scheme, based primarily on functional criteria, represented a useful classification framework, but with the widespread use of radioligands and second messenger systems in the mid 1980's, subtypes of 5-HT_1 receptor binding sites were described; and it became rapidly obvious that the 5-HT_{1C} receptor would be better classified within the 5-HT_2 family, suggesting 5-HT_2 subtypes also. A novel 5-HT receptor was identified in the gastrointestinal (G. I.) tract and brain, termed 5-HT_4. In 1988 then, the molecular biology era started with the cloning of the 5-HT_{1A} receptor. Soon, most known or suspected 5-HT receptors were cloned in close succession. This work led to the identification of a number of 'new' receptors, without obvious physiological counterparts. Tentatively termed 5-ht_{1E}, 5-ht_{1F}, 5-ht_{5A}, 5-ht_{5B}, 5-ht_6, 5-HT_7, they required integration into the classification. Thus, the Serotonin Club Receptor Nomenclature Committee proposed a new classification system based on operational, structural and transductional information (Humphrey et al. 1993). These principles were subsequently applied to additional receptor families by the receptor Nomenclature Committee of the International Union of Pharmacology (NC-IUPHAR). The current classification (Hoyer et al. 1994) is progressively adapted to incorporate new information, obtained with both recombinant and native receptors, and favours an alignment of nomenclature with the human genome to avoid species differences (see Hartig et al. 1996; Hoyer and Martin 1997). Currently, seven families of 5-HT receptors have been recognised. A graphical representation of the current classification of 5-HT receptors is given in Figure 1.

5-HT_1 receptors

The 5-HT_1 receptor class comprises five receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-ht_{1E} and 5-ht_{1F}) which, in humans, share 40-63 % overall sequence identity and couple somewhat preferentially to $G_{i/o}$ to inhibit cAMP formation (see Tables 1 and 2). The 5-ht_{1E} and 5-ht_{1F} receptors are given a lower case appellation to denote that endogenous receptors with a physiological role have not yet been found. In contrast, 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors have been demonstrated functionally in a variety of tissues. The 5-HT_{1C} designation is vacant, as the receptor was renamed 5-HT_{2C} due to structural, operational and transductional similarities with the 5-HT_2 receptor subclass (Hoyer et al., 1994).

5-HT_{1A} receptors

The human 5-HT_{1A} receptor is located on chromosome 5q11.2-q13. 5-HT_{1A} receptors are largely distributed throughout the CNS. In the raphé nuclei, they are somatodendritic and act as autoreceptors to inhibit cell firing; postsynaptic 5-HT_{1A} receptors are present in limbic structures, particularly the hippocampus. 5-HT_{1A} receptors mediate neuronal hyperpolarisation, via G-protein coupled K^+ channels. In the G.I. tract, 5-HT_{1A} receptors on the guinea pig myenteric plexus act as inhibitory modulators of fast excitatory postsynaptic potentials.

5-HT_{1A} receptors have been implicated in the neuroendocrine regulation of adrenocorticotrophic hormone (ACTH) secretion. Activation of postsynaptic 5-HT_{1A} receptors induces a behavioural syndrome: flat body posture, reciprocal forepaw treading and head weaving. The sponta-

neous tail-flick response has also been attributed to postsynaptic 5-HT_{1A} receptor activation, whereas evidence for a presynaptic 5-HT_{1A} (auto)receptor in the hyperphagic response appears convincing. The hypothermic response to 5-HT_{1A} agonists in the rat implies both pre- and postsynaptic mechanisms. A decrease in blood pressure and heart rate, and increased locomotor responses can be induced by central 5-HT_{1A} receptor activation. The proposed role of 5-HT_{1A} receptors in modulating anxiety-related behaviours is supported by recent studies utilising 5-HT_{1A} receptor knock-out (KO) mice. They display increased anxiety in a number of tests (e.g. elevated plus maze, elevated zero maze, open field). Moreover, they show decreased baseline immobility in the forced swim and tail suspension tests.

5-HT_{1A} receptor agonists, such as buspirone or gepirone, are being used/developed for the treatment of anxiety and depression. Furthermore, the 5HT_{1A} receptor and beta adrenoceptor antagonist, pindolol, was reported to enhance the therapeutic efficacy, and shorten the onset of action of SSRIs, upon co-administration in severely depressed patients. However, both positive and negative findings have been reported, as is common in depression trials. Flesinoxan, a 5-HT_{1A} receptor agonist, was initially developed as an antihypertensive agent, however its effects in patients were disappointing and this approach has now been abandoned.

Several agonists show selectivity for the 5-HT_{1A} receptor, particularly 8-hydroxy-di-n-propylamino tetralin (8-OH-DPAT), a full agonist in most systems, whilst the anxiolytics, buspirone and gepirone, and other ligands such as MDL 72832 are partial agonists. To date, the only selective high affinity silent antagonist at this receptor is WAY 100635. The agonists U-92016A and (+)UH 301 and the antagonists, (-) UH 301 and NAD 299 are other tools of interest.

5-HT_{1B} receptors

The 5-HT_{1B} and 5-HT_{1D} receptors have experienced a complex and debated history. The 5-HT_{1B} receptor was originally defined according to operational criteria (high affinity for some indole blockers) and was thought to be a rodent specific receptor, whereas the 5-HT_{1D} receptor was found in other species (similar distribution and function, but different "pharmacology"). It turned out that in all species investigated, two related receptors could be cloned (5-HT_{1B} and 5-HT_{1D}). The differences in the pharmacology of the 5-HT_{1B} receptor across species are attributable to the mutation of a single amino acid Asp^{123} to Arg^{123}. The human 5-HT_{1B} receptor is located on chromosome 6q13.

5-HT_{1B} receptors are expressed in the CNS, in the basal ganglia, striatum and frontal cortex and are thought to serve as terminal autoreceptors. The receptor may also act as a terminal heteroreceptor controlling the release of other neurotransmitters, such as acetylcholine, glutamate, dopamine, noradrenaline and γ-aminobutyric acid. Additional effects tentatively attributed in rats to central 5-HT_{1B} receptor activation, include hyperlocomotion, hypophagia, hypothermia and penile erection. The receptors are also found on cerebral arteries and other vascular tissues. 5-HT_{1B} receptors mediate contraction of rat caudal arteries. Other 5-HT_{1B} effects include inhibition of noradrenaline release in vena cava and inhibition of plasma extravasation produced by trigeminal ganglion stimulation in guinea pigs and rats.

Interest in 5-HT_{1B} receptor agonists has been triggered by the anti-migraine properties of sumatriptan, a non-selective 5-$HT_{1D/1B}$ receptor agonist; various agonists have been developed for this indication (dihydroergotamine (DHE), zolmitriptan, naratriptan, rizatriptan, elitriptan, almotriptan, donitriptan and others). The putative 5HT_{1B} receptor agonist, anpirtoline, has analgesic and antidepressant-like properties in rodents and interestingly 5-HT_{1B} receptor KO mice were reported to be highly aggressive and show an increased preference for alcohol. In contrast to the 5-HT_{1A} receptor KO mouse, the 5-HT_{1B} receptor KO has a different, and in most cases opposite behavioural profile, displaying decreases in measures of anxiety in the elevated plus maze, open field and tail suspension tests, in addition to an increase in aggression in the resident intruder paradigm. However, the development of 5-HT_{1B} agonist 'serenics' such as eltoprazine was not successful; the expected anti-aggressive effects were not observed in patients.

Selective 5-HT_{1B} agonists include MK 462 (rizatriptan), BW 311C90 (zolmitriptan), SKF 99101H, GR 46611, L 694247, and CP 93129 (in rodents). However, some of them, e.g. sumatriptan or LY 334370 have significant affinity to 5-ht_{1F} receptors. Clearly, some of these molecules will recognise 5-HT_{1B} and 5-HT_{1D} receptors almost equally, e.g. L 694247. However, SB 216641 (h5-HT_{1B}) and BRL 15572 (h5-HT_{1D}) have permitted discrimination of the effects mediated by one or the other of these receptors, in appropriate species, at the level of presynaptic auto- and heteroreceptors. Of 5-HT_{1B} receptor antagonists, the most commonly used (in rodents), pindolol, cyanopindolol and SDZ 21009 are equipotent at the 5-HT_{1A} receptor, where they have antagonist or partial agonist properties, (and are potent beta-adrenoceptor antagonists). SB 216641, SB 272183 and GR 55562 demonstrate a certain degree of 5-HT_{1B} selectivity. SB 224289 and SB 236057 are inverse agonists, allowing the characterisation of 5-HT_{1B} receptor tone. These new compounds confirm that terminal 5-HT autoreceptors are of the 5-HT_{1B} type.

Radiolabelled ligands include [^{3}H]-GR 125743, a 5-$HT_{1D/1B}$ receptor antagonist, [^{125}I]5-hydroxytryptamine-5-*O*-carboxymethylglycyltyrosinamide (GTI) or [^{3}H]alniditan. Finally, in rodents, [^{125}I]cyanopindolol under appropriate conditions labels 5-HT_{1B} sites.

5-HT$_{1D}$ receptors

The 5-HT$_{1D}$ receptor is located on chromosome 1p34.3-p36.3 and possesses 63 % overall structural homology with the 5-HT$_{1B}$ receptor. Its level of expression is very low compared with 5-HT$_{1B}$ receptors and it has been difficult to assign a functional role. The characteristics of the 5-HT$_{1B}$ and 5-HT$_{1D}$ subtypes are now well established, and the use of the new 5-HT$_{1B/1D}$ selective ligands, SB 216641 (h5-HT$_{1B}$) and BRL 15572 (h5-HT$_{1D}$), suggests the presence of a 5-HT$_{1D}$ autoreceptor in the dorsal raphé nuclei. 5-HT$_{1D}$ receptors in human heart modulate 5-HT release. The currently available anti-migraine drugs do not distinguish between 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors. However, the selective 5-HT$_{1D}$ receptor agonist, PNU 109291, has been shown to play a significant role in the suppression of meningeal neurogenic inflammation and trigeminal nociception in guinea pig, suggesting the 5-HT$_{1D}$ receptor related headaches, but such drugs are devoid of vascular activity confirming that it is the 5-HT$_{1B}$ receptor that mediates the vasoconstrictor effects produced by sumatriptan and analogues. Both 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor immunoreactivity is found in human trigeminal ganglia, where the receptors colocalise with calcitonin gene-related peptide, substance P and nitric oxide synthase.

5-ht$_{1E}$ receptors

The putative 5-ht$_{1E}$ receptor was identified in binding studies in human frontal cortex, but its overall distribution is still to be described due to the absence of adequate tools. It is a 365 amino acid protein negatively linked to adenylyl cyclase in recombinant cell systems, present on human chromosome 6q14-q15. 5-ht$_{1E}$ receptor mRNA and recognition sites exhibiting the pharmacological characteristics of the receptor have been mapped in rodent and human brain. However, confirmation of a true physiological role for 5-ht$_{1E}$ receptors is still lacking; hence, they retain their lower case appellation. A thorough characterisation of the 5-ht$_{1E}$ receptor awaits the development of selective ligands.

5-ht$_{1F}$ receptors

The 5-ht$_{1F}$ receptor located on chromosome 3p11, has 366 amino acids, is negatively linked to adenylyl cyclase in recombinant cell systems, and most closely related to the 5-ht$_{1E}$ receptor with > 70 % sequence homology across the seven transmembrane domains. Little is known about the distribution and function of the 5-ht$_{1F}$ receptor; mRNA for the human receptor protein has been identified in the brain (dorsal raphé, hippocampus, cortex, striatum, thalamus and hypothalamus), mesentery and uterus. The anti-migraine 5-HT$_{1B/1D}$ agonist sumatriptan labels 5-ht$_{1F}$ sites with high affinity. The binding site distribution obtained was very similar to that for 5-ht$_{1F}$ mRNA. Naratriptan also has affinity for 5-ht$_{1F}$ receptors and it has been hypothesised that they might be a target for anti-migraine drugs. 5-ht$_{1F}$ receptor mRNA has been detected in the trigeminal ganglia, stimulation of which leads to plasma extravasation in the dura, a component of neurogenic inflammation thought to be a possible cause of migraine. LY 334370, a selective 5-ht$_{1F}$ receptor agonist, inhibits trigeminal stimulation-induced early activated gene expression in nociceptive neurones in the rat brainstem. LY 334370, as a radioligand, shows prominent binding in the cortical areas, striatum, hippocampus and olfactory bulb compatible with 5-ht$_{1F}$ mRNA distribution.

5-ht$_{1F}$ selective ligands i.e. LY 344864 and BRL 54443, are currently in development (migraine), however they also have affinity for 5-ht$_{1E}$ receptors.

5-HT$_2$ receptors

5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors exhibit 46-50 % overall sequence identity and couple preferentially to $G_{q/11}$ to increase inositol phosphates and cytosolic [Ca^{++}] (see Tables 1 and 3). The 5-HT$_{2A}$ receptor refers to the classical D receptor initially described by Gaddum and Picarelli (1957), and later defined as the 5-HT$_2$ receptor by Peroutka and Snyder (1979). 5-HT$_{2B}$ receptors mediate the contractile action of 5-HT in the rat fundus. In human pulmonary artery endothelial cells, 5-HT$_{2B}$ receptor stimulation causes intracellular calcium release. The third 5-HT$_2$ subtype corresponds to the previously known 5-HT$_{1C}$ receptor, which was reclassified as 5-HT$_{2C}$ (Hoyer et al. 1994).

5-HT$_{2A}$ receptors

The 5-HT$_{2A}$ receptor located on human chromosome 13q14-q21, has 471 amino acids in rat, mouse and humans. It is widely distributed in peripheral and central tissues. 5-HT$_{2A}$ receptors mediate contractile responses in many vascular and non-vascular smooth muscle preparations, e.g. bronchial, uterine, ileal and urinary smooth muscle. 5-HT$_{2A}$ receptors mediate platelet aggregation and increased capillary. Centrally, these receptors are principally located in the cortex, claustrum and basal ganglia. 5-HT$_{2A}$ receptor activation stimulates hormone secretion e.g. ACTH, corticosterone, oxytocin, renin, and prolactin. 5-HT$_2$ receptor agonists, in addition to precursors of 5-HT and 5-HT releasing agents, produce head twitching in mice, and wet-dog shakes and back muscle contractions in rats. Truly selective agonists have not been described, since aMe-5-HT, DOI, DOM and DOB all recognise other receptors of the 5-HT$_2$ receptor class. The production of drug discriminative stimulus properties to 5-HT$_2$ receptor agonists, e.g. (-)2,5,-dimethoxy-4-methamphetamine (DOM) can be blocked by 5-HT$_2$ receptor antagonists such as ketanserin. LSD and other hallucinogens most probably produce hallucinations via 5-HT$_{2A}$ receptors. Although their selectivity *vis a vis* 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors is rather limited, this represents currently the best possible explanation.

Table 2. 5-$HT_{2, 3, 4}$ receptor nomenclature proposed by the NC-IUPHAR Subcommittee on 5-HT receptors.

Nomenclature	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}*	5-HT_3	5-HT_4
Previous names	D / 5-HT_2	5-HT_{2F}	5-HT_{1C}	M	
Selective agonists	DOI†	BW 723C86	Ro 600175	SR 57227 *m*-chlorophenyl-biguanide	BIMU 8 RS 67506 ML 10302
Selective antagonists (pK_B)	Ketanserin (8.5-9.5) MDL 100907 (9.4)	SB 200646 (7.5)†† SB 204741 (7.8)	Mesulergine (9.1) SB 242084 (9.0) RS 102221 (8.4)	granisetron (10) ondansetron (8-10) tropisetron (10-11)	GR 113808 (9-9.5) SB 204070 (10.8) RS 100235 (11.2)
Radioligands	[^{125}I]DOI [^{3}H]Ketanserin [^{3}H]MDL 100907	[^{3}H]5-HT	[^{125}I]LSD [^{3}H]Mesulergine	[^{3}H]*(S)*-zacopride [^{3}H]tropisetron [^{3}H]granisetron [^{3}H]GR 65630 [^{3}H]LY 278584	[^{125}I]SB 207710 [^{3}H]GR 113808 [^{3}H]RS 57639
G protein effector	$G_{q/11}$	$G_{q/11}$	$G_{q/11}$	§	G_s
Gene/Chromosomal localisation	*HTR2A*/13q14-q21	*HTR2B*/2q36.3-q37.1	*HTR2C*/Xq24	*HTR3*/11q23.1-q23.2	*HTR4*/5q31-33
Structural information	h471 P28223 m471 P35362 r471 P14842	h481 P41595 m504 Q02152 r479 P30994	h458 P28335 m459 P34968 r460 P08909	Multi-subunit¶ 5-HT_{3A}, 5-HT_{3B}, 5-ht_{3C}	h387 Y09756[AS] m387 Y09587[AS] r387 U20906[AS]

†Also activates the 5-HT_{2C} receptor. ††Nonselective blockade. *Multiple isoforms of the 5-HT_{2C} receptor are produced by RNA editting. §The 5-HT_3 receptor is a transmitter-gated cation channel that exists as a pentamer of 4TM subunits. ¶Human, rat, mouse, guinea-pig and ferret homologues of the 5-HT_{3A} receptor have been cloned that exhibit interspecies variation in pahrmacology. A second 5-HT_3 receptor subunit, 5-HT_{3B}, imparts distinctive biophysical properties upon hetero-oligomeric (5-HT_{3A}/5-HT_{3B}) versus homo-oligomeric (5-HT_{3A}) recombinant receptors.

Ketanserin and MDL 100907 are selective antagonists. Ketanserin was developed for the treatment of hypertension, but 5-HT_{2A} receptor antagonism as a valid anti-hypertensive principle is not questioned, since ketanserin is a potent α_1 adrenoceptor antagonist. 5-HT_{2A} receptor antagonists such as risperidone, ritanserin, seroquel, olanzapine or MDL 100907 have been indicated/developed for the treatment of schizophrenia. However, development of MDL 100907 for acute schizophrenia was stopped. The combination of dopamine D_2 and 5-HT_{2A} receptor antagonism may still explain the anti-psychotic activity of drugs such as clozapine, olanzapine, seroquel and others.

5-HT_{2B} receptors

The 5-HT_{2B} receptor mediates fundic smooth muscle contraction. It was difficult to characterise pharmacologically, due to operational features similar to those of other members of the 5-HT_2 family (Humphrey et al. 1993). Eventually, the cloning of the rat, mouse and human 'fundic' receptors (also known as 5-HT_{2F}) clarified the issue. It is located on human chromosome 2q36.3-2q37.1. 5-HT_{2B} receptor mRNA is found in rat fundus, gut, heart, kidney, lung and brain. Centrally, 5-HT_{2B} receptor-like immunoreactivity is restricted to cerebellum, lateral septum, hypothalamus and medial amygdala. Application of BW 723C86 into the medial amygdala produces anxiolytic properties in rat social interactions. 5-HT_{2B} receptor activation has been implicated in mediating hyperphagia. 5-HT_{2B}, but not 5-HT_{2C}, receptor mRNA is found in a number of blood vessels. 5-HT_{2B} receptors on endothelial cells of pig pulmonary arteries and in rat jugular vein mediate vasorelaxation via NO release. 5-HT_{2B} receptors contract longitudinal muscle in human small intestine and when expressed in mouse fibroblast cells, cause mitogenesis linked to tumour transforming activity, via MAP kinase activation.

SB 204741 was the first selective 5-HT_{2B} receptor antagonist. SB 200646 and SB 206553 have been reported as selective 5-$HT_{2C/2B}$ receptor antagonists, with low affinity for 5-HT_{2A} and other sites. BW 723C86 has agonist selectivity at the rat 5-HT_{2B} receptor, although less marked at human receptors. 5-HT_{2B} receptor antagonists such as SB 200646 may be indicated for the treatment of migraine prophylaxis, given the vasodilatatory role of this receptor and that a number of 'older' antimigraine drugs share 5-HT_{2B} receptor antagonism. Activation of the 5-HT_{2B} receptor is most probably responsible for the valvulopathies reported for appetite suppressant preparations containing dex-fenfluramine.

5-HT_{2C} receptors

The 5-HT_{2C} receptor was mapped to human chromosome Xq24. Given its similar pharmacological and transductional features with the 5-HT_{2A} receptor, the sequence of the latter was established by homology cloning based on the 5-HT_{2C} sequence. However, due to the lack of truly selective 5-HT_{2C} receptor ligands, our current knowledge concerning a func-

tional role of this receptor is still rather limited. Thus far, its distribution has been limited to the CNS and choroid plexus, where the receptor was originally identified. 5-HT_{2C} receptors in the choroid plexus couple to PLC activity, but additional functional correlates remain largely to be found. At least fourteen functional 5-HT_{2C} receptor isoforms are produced by adenine deaminase editing of the receptor mRNA.

MK 212 and Ro 600175 are moderately selective 5-HT_{2C} agonists; amongst the antagonists, LY 53857, ZM 170809, ritanserin, mianserin and mesulergine have been utilised, but are essentially non-selective. The anxiogenic component of mCPP may be mediated by 5-HT_{2C} receptor activation, and selective 5-HT_{2C} receptor antagonists such as SB 242084 display anxiolytic properties in various animal models. However, additional studies utilising selective agonists are required (e.g. Ro 600175). mCPP or Ro 600175 cause additional behavioural responses attributed to central 5-HT_{2C} receptor activation, e.g. hypoactivity, hypophagia, increased penile grooming/erections and oral dyskinesia. 5-HT_{2C} receptor activation produces a tonic, inhibitory influence upon frontocortical dopaminergic and adrenergic, but not serotonergic transmission and, in part, to play a role in neuroendocrine function. RS 102221 increased food-intake and weight gain in rats, but failed to reverse the mCPP induced hypolocomotion, possibly due to restricted brain penetration. 5-HT_{2C} receptor KO mice have spontaneous convulsions, cognitive impairment, increased food intake and obesity, but similar effects are not reproduced by selective antagonists, suggesting that these changes may result in part from neuroadaptation. Nevertheless, the 5-HT_{2C} receptor is an attractive target for the discovery of novel treatment for feeding disorders.

5-HT_3 receptors

5-HT_3 receptors (M receptors of Gaddum and Picarelli 1957) belong to the ligand-gated ion channel receptor superfamily, similar to the nicotinic acetylcholine or $GABA_A$ receptors and share electrophysiological and structural patterns. The receptors are found on central and peripheral neurones, where they trigger rapid depolarisation due to the opening of non-selective cation channels (Na^+, Ca^{++} influx, K^+ efflux). The response desensitises and resensitises rapidly. The native 5-HT_3 receptor, as revealed by electron microscopy in neuroblastoma-glioma cells, is a pentamer.

A cDNA encoding a single subunit of the 5-HT_{3A} receptor was isolated from a neuronally derived cell line. The human homologue maps to chromosome 11q23.1-q23.2. Two splice variants were reported in neuroblastoma-glioma cells (NCB-20, NG 108-15) and rat native tissue, with similar distribution, pharmacological profiles and electrophysiological characteristics when expressed as homomers. 5-HT_3 receptors are present in the CA1 pyramidal cell layer in the hippocampus, the dorsal motor nucleus of the solitary tract and the area postrema. In the periphery, they are located on pre- and postganglionic autonomic neurones and on neurones of the sensory nervous system. 5-HT_3 receptor activation has pronounced effects on the cardiovascular system and regulates both motility and intestinal secretion throughout the entire G.I. tract. Species differences provide the basis of the pharmacological heterogeneity reported thus far. But, after extensive investigation, a second subunit, 5-HT_{3B}, was cloned. The heteromeric combination of 5-HT_{3A} and 5-HT_{3B} subunits provides the full functional features of the 5-HT_3 receptor; since the 5-HT_{3A} subunit alone results in receptors with very low conductance and response amplitude, whereas 5-HT_{3B} homomers have no activity. The patent literature has recently reported the cloning of a third subunit, 5-ht_{3C}, but no details are presently available on its features. The 5-HT_3 receptor, like other members of the ligand-gated ion channel receptor superfamily, possesses additional, pharmacologically distinct, recognition sites, subject to allosterical modulation.

5-HT_3 antagonists ondansetron, granisetron and tropisetron are used clinically in chemotherapy- and radiotherapy-induced nausea and vomiting. Since 5-HT_3 receptor activation in the brain leads to dopamine release, and 5-HT_3 receptor antagonists produce central effects comparable to those of anti-psychotics and anxiolytics; schizophrenia and anxiety were considered as potential indications. 5-HT_3 receptor antagonists have been reported to induce cognition enhancing effects. However, there are not enough clinical data to substantiate such activities. Similarly, that 5-HT_3 antagonists should prove useful in the treatment of migraine did not materialise in clinical studies. More recently, alosetron was developed for the treatment of women suffering from IBS with diarrhoea, had to be withdrawn due to safety reasons, but has been accepted again by the FDA with some label restrictions.

5-HT_4 receptors

5-HT_4, 5-ht_6 and 5-HT_7 receptors all couple preferentially to G_s and promote cAMP formation, yet they are classified as distinct receptor classes because of their limited (< 35 %) overall sequence identities. This subdivision is arbitrary and may be subject to future modification.

The 5-HT_4 receptor had been well described in both central and peripheral tissues long before cloning, although confusion between 5-HT_3 and 5-HT_4 receptors occurred at times. Their existence was reported in rat neonatal colliculi over 20 years ago, but they were not properly identified at the time: the 5-HT4 receptor was really charaterised by Bockaert and colleagues using substituted benzamide derivatives like cisapride, renzapride or zacopride, acted as agonists at the 'atypical' 5-HT receptor in mouse colliculi stimulation cAMP. Interestingly, the potent 5-HT_3 receptor antagonist tropisetron

Table 3. 5-$HT_{5, 6, 7}$ receptor nomenclature proposed by the NC-IUPHAR Subcommittee on 5-HT receptors.

Nomenclature	5-ht_{5A}	5-ht_{5B}	5-ht_6	5-HT_7
Previous names	5-$HT_{5\alpha}$	-	-	5-HT_x 5-HT_1-like
Selective agonists	-	-	-	-
Selective antagonists (pK_B)	-	-	Ro 630563 (7.9) SB 271046 (7.8) SB 357134 (8.5)	SB 258719 (7.9) SB 269970 (9.0)
Radioligands	[^{125}I]LSD [^{3}H]5-CT	[^{125}I]LSD [^{3}H]5-CT	[^{125}I]SB 258585 [^{125}I]LSD [^{3}H]5-HT	[^{125}I]LSD [^{3}H]SB 269970 [^{3}H]5-CT [^{3}H]5-HT
G protein effector	$G_{i/o}$	None identified	G_s	G_s
Gene/Chromosomal localisation	*HTR5A*/7q36.1	*htr5b*/2q11-q13	*HTR6*/1p35-36	*HTR7*/10q23.3-24.3
Structural information	h357 P47898 m357 P30966 r357 P35364	m370 P31387 r370 P35365	h440 P50406 m440 NP_067333 r438 P31388	h445 P34969[AS] m448 P32304 r448 P32305[AS]

(ICS 205-930) was described as the first competitive 5-HT_4 receptor antagonist. The human 5-HT_4 receptor was mapped to chromosome 5q31-33. At least seven C-terminal splice variants of the receptor have been identified (5-HT_{4a}-5-HT_{4h}), in addition to a novel splice variant, 5-HT_{4hb}, with a 14 amino acid insertion in the second extracellular loop.

The 5-HT_4 receptor variants couple positively to adenylyl cyclase and share pharmacological profiles. One important feature of the receptor is the level of constitutive activity, which is expressed at rather low receptor levels, which may well explain differences observed with respect to variable intrinsic activity of a number of ligands, depending on tissue and/or species. The pattern of expression of the human 5-HT_4 receptor isoforms is tissue specific. The h5-HT_{4d} receptor isoform has not yet been described in other species. It is limited to the gut, whereas the other isoforms are more widely expressed. In addition to adenylate cyclase stimulation, direct coupling to potassium channels and voltage-sensitive calcium channel have been proposed.

The receptor is labelled with [^{3}H]GR 113808, [^{3}H]RS 57639 and [^{125}I]SB 207710. In the brain, the distribution of radioligand binding sites and mRNA is similar. RT-PCR studies have also demonstrated 5-HT_4 receptor mRNA is present in vascular smooth muscle, confirming functional studies. 5-HT_4 receptor activation triggers acetylcholine release in the guinea-pig ileum and contracts the oesophagus and colon. The 5-HT_4 receptor stimulates secretory responses to 5-HT in intestinal mucosa. Electrogenic ion transport is stimulated through 5-HT_4 receptors in the small intestine; whilst in piglet heart the receptors mediate tachycardia (right atria) and positive inotropic effects (left atria). Similarly, isolated human atrial appendages respond with increased contractile force to 5-HT_4 receptor agonists. 5-HT_4 receptors in the CNS appear to modulate neurotransmitter (acetylcholine, dopamine, serotonin and GABA) release, enhance synaptic transmission and may play a role in memory enhancement; confirmation by clinical studies is lacking though. Desensitisation is seen in many experimental in vitro models, though readily reversible upon agonist removal in G.I. tract preparations.

Potent and selective 5-HT_4 receptor ligands are available, e.g. the agonists BIMU 8, RS 67506 and ML 10302, and the antagonists GR 113808, SB 204070, SB 203186, RS 23597-190 and RS 39604; which should allow definition of the (patho)physiological role of this receptor. Selective 5-HT_4 receptor ligands may have therapeutic utility in a number of disorders, including cardiac arrhythmia, neurodegenerative diseases and urinary incontinence. Cisapride, a gastroprokinetic agent, acts as an agonist at the 5-HT_4 receptor. Tegaserod (HTF-919, Zelmac/Zelnorm), a new generation 5-HT_4 receptor partial agonist, is used to treat constipation predominant IBS, and its therapeutic activity in functional motility disorders of the upper G.I. tract is currently under clinical investigation.

5-ht_5 receptors

Two subtypes of the 5-ht_5 receptor (5-ht_{5A} and 5-ht_{5B}), sharing 70 % overall sequence identity, have been found in rodents, whereas the 5-ht_{5A} subtype found in humans, was mapped to chromosome 7q36.1. The human 5-ht_{5B} receptor gene does not encode a functional protein, due to the presence of stop codons in its coding sequence. Human recombinant 5-ht_{5A} receptors inhibit forskolin stimulated cAMP production, although the receptor may also couple positively to cAMP. In *Xenopus* oocytes, the human 5-ht_{5A} receptor couples to the inwardly rectifying K^+ channel, $GIRK_1$.

5-ht_5 mRNA is present in hypothalamus, hippocampus, corpus callosum, fimbria, cerebral ventricles and glia, and a role has been sugested in reatcive gliosis. A physiological functional response or specific 5-ht_5 binding are still missing.

5-ht_6 receptors

The rat 5-ht_6 receptor has 438 amino acids and is positively coupled to adenylyl cyclase via G_s. The human gene has 89 % sequence homology with its rat equivalent, and maps chromosome region 1p35-p36. Rat and human 5-ht_6 receptor mRNA is located in the striatum, amygdala, nucleus accumbens, hippocampus, cortex and olfactory tubercle, but has not been found in peripheral organs. Circumstantial evidence suggests the 5-ht_6 receptor to be expressed endogenously in neuronal tissue.

The 5-ht_6 receptor promotes accumulation of cAMP. NCB 20 and N18TG2 cells and rat striatal neuronal cultures express a receptor which couples positively to adenylyl cyclase and displays an operational profile consistent with the recombinant 5-ht_6 receptor. Similar evidence for the putative 5-ht_6 receptor has been obtained in homogenates of pig caudate nucleus: cAMP accumulation had a 5-ht_6 receptor profile and was antagonised by clozapine and methiothepin. [^{3}H]clozapine binds with nanomolar affinity to two distinct sites in rat brain; one site displays the operational 5-ht_6 receptor profile.

SB 271046 is a potent, selective, and bioavailable 5-ht_6 receptor antagonist; EMDT is a selective 5-ht_6 receptor agonist. The 5-ht_6 receptor is labelled with [^{125}I]SB 258585. Intracerebroventricular injections of 5-ht_6 receptor antisense oligonucleotides gave rise to a specific behavioural syndrome of yawning, stretching and chewing and caused a 30 % reduction in the number of [^{3}H]LSD binding sites (measured in the presence of 300 nM spiperone). The antisense-induced behavioural syndrome can be dose-dependently antagonised by atropine, implying a modulatory role for 5-ht_6 receptors on cholinergic neurones. The selective 5-ht_6 receptor antagonist, Ro 04-6790, produces a behavioural syndrome involving an increase in acetylcholine neurotransmis-sion. Enhanced retention of spatial learning following both antisense oligonucleotides and Ro 04-6790 have been reported. A role for the 5-ht_6 receptor in the control of central cholinergic function, and thus a putative target for cognitive dysfunction such as Alzheimer's disease is thus suggested. In addition, antisense oligonucleotide treatment reduced both food consumption and body weight; the later effect was also seen following Ro 04-6790 suggesting potential in feeding disorders.

Antipsychotics (clozapine, olanzapine, fluperlapine and seroquel) and antidepressants (clomipramine, amitryptyline, doxepin and nortryptyline) are 5-ht_6 receptor antagonists, in addition to multiple other affinities. This attribute tempted speculation of an involvement of the 5-ht_6 receptor in psychiatric disorders.

5-HT_7 receptors

The 5-HT_7 receptor gene is located on human chromosome 10q23.3-q24.4. It has 445 amino acids and positively modulates cAMP formation via G_S. The receptor shares a low homology with other members of the 5-HT receptor family (< 50 %). The receptor also activates the mitogen-activated protein kinase, ERK, in primary neuronal cultures. The cDNA encoding the receptor contains two introns; one located in the second intracellular loop, the other in the predicted intracellular carboxyl terminal. Alternate splicing of this latter intron has been reported to generate four 5-HT_7 receptor isoforms (5-HT_{7a}-5-HT_{7d}), which differ in their C-termini. The various isoforms have similar pharmacology, signal transduction and to a lesser extent tissue distribution. The 5-HT_7 receptor has high affinity for the prototypical 5-HT_1 agonists 5-CT, 5-MeOT and 8-OH-DPAT, the 5HT_2 receptor ligand LSD and the antagonists, ritanserin, metergoline, methysergide and mesulergine. It has an extensive vascular distribution, and is responsible for the prominent, persistent vasodilator response to 5-HT in anaesthetised animals, and is also expressed in non-vascular smooth muscle and the CNS.

[^{3}H]5-CT, in the presence of (-)-cyanopindolol and sumatriptan, labels 5-HT_7 recognition sites in guinea pig cerebral cortex membranes. Autoradiographic analysis revealed binding sites in the medial thalamic nuclei and related limbic and cortical regions of the guinea pig brain, with lower densities in the sensory relay nuclei, substantia nigra, hypothalamus, central grey and dorsal raphé nuclei, in agreement with 5-HT_7 receptor mRNA. In rat suprachiasmatic (SCN) neurones, a gamma-aminobutyric acid activated current (I_{GABA}) is inhibited by 5-HT, consistent with the presence of 5-HT_7 receptors in the SCN. Moreover, the cellular localisation of rat hypothalamic 5-HT_7 receptors was suggested to be postsynaptic, with respect to serotonergic neurones, and regulated by altered synaptic levels of endogenous neurotransmitter.

The presence of 5-HT_7 sites in the limbic system and thalamocortical regions, suggest a role in the affective disorders; indeed, atypical antipsychotics, e.g. clozapine, risperidone and antidepressants have high affinity for the 5-HT_7 receptor. 5-HT_7 receptor down-regulation occurs after chronic antidepressant treatment, and acute, but not chronic, stress regulates 5-HT_7 receptor mRNA expression.

The antagonists SB 258719 and SB 269970 are selective. A role for the 5-HT_7 receptor has been proposed in the 5-CT-induced hypothermia in guinea pigs which is blocked by both SB 269970 and the non-selective 5-HT_7 receptor antagonist, metergoline. SB 269970 significantly reduced time spent in paradoxical sleep in rats. [^{3}H]SB 269970 is a selective radioligand for 5-HT_7 receptors. The 5-HT_7 receptor is the orphan known as the '5-HT_1-like' receptor mediating relaxation of the guinea pig isolated ileum and cat saphenous vein

and subsequently shown to mediate elevation of cAMP and relaxation in neonatal porcine vena cava.

Putative orphan 5-HT receptors

Several endogenous 5-HT receptors have been defined phamacologically, although a corresponding gene product encoding the receptor has yet to be identified. As long as their structure is unknown, these receptors are regarded as orphans in the current nomenclature. One of these however, the so-called '5-HT_1-like' receptor mediating direct vasorelaxation corresponds to the 5-HT_7 receptor (see above). On the other hand, the situation with the remaining orphan receptors (see Hoyer et al. 1994) has not evolved further and thus the *status quo ante* remains. In particular, no progress has been made with the 5-HT_{1P} receptor, which is present in the gut and whose pharmacology is reminiscent of the 5-HT_4 receptors, with the restriction that some of the ligands described, like the 5-HT dipeptides, do not affect 5-HT_4 receptors. Also, a high affinity binding site for [^{3}H]5-HT with novel '5-HT_1-like' pharmacology has been reported in mammalian brain, but has yet to be sufficiently characterised for inclusion in the 5-HT_1 receptor family.

Conclusion

Whether the almost endless diversity in 5-HT receptors and transporters fulfils specific physiological and/or pathophysiological roles is an open question. However, it may soon be possible to determine which form of a given receptor is expressed in a given tissue. This will then assist in designing drugs with an adequate profile at the target organ, assuming it is known. The diversity in receptors described here suggests that under physiological and more so under pathological conditions, the status of the receptors may vary dramatically from one subject to another, explaining differences in responder rates to a given treatment. It is clear that receptor cross-talk will considerably affect the responsiveness of one patient versus another. Indeed, vascular reactivity towards triptans varies significantly between patients possibly because of such possibilities. Depending on the nature of the receptor isoforms (5-HT_4, 5-HT_7 or 5-HT_{2C}) expressed in the G.I. tract/vessel/brain, it could be anticipated that certain patients may demonstrate enhanced responsivity to particular treatments, i.e. titration may represent a rule, rather than an exception. The human genome being almost completely sequenced, we will know soon whether there are more 5-HT receptors. However, given the interactions with accessory proteins, in addition to homo- and heterodimerisation, one can predict that the situation will not prove simpler in the short term.

Drugs

5-CT: 5-carboxamidotryptamine

8-OH-DPAT: 8-hydroxy-2-(di-*n*-propylamino)tetralin

BIMU 8: (endo-*N*-8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-3-isopropyl-2-oxo-1*H*-benzimidazol-1-carboxamide hydrochloride

BRL 15572: 3-[4-(3-chlorophenyl) piperazin-1-yl]-1,1,-diphenyl-2-propanol

BRL 54443: 3-(1-methylpiperidin-4-yl)1*H*-indol-5-ol

BW 311C90: (S)-4-[[3-[2-(Dimethylamino)ethyl]-1H-indol-5-yl]methyl]-2-oxazolidinone

BW 723C86: 1-[5(2-thienylmethoxy)-1*H*-3-indolyl]propan-2-amine hydrochloride

CP 93129: 5H-Pyrrolo[3,2-b]pyridin-5-one, 1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)

DOB: 2,5-dimethoxy-4-bromoamphetamine

DOI: 2,5-dimethoxy-4-iodoamphetamine

EMDT: 2-ethyl-5-methoxy-N,N-dimethyltryptamine

GR 113808: [1-2[(methylsuphonyl)amino]ethyl]-4-piperidinyl]methyl-1-methyl-1*H*-indole-3-carboxylate

GR 125743: *n*-[4-methoxy-3-(4-methyl-1-piperizinyl)phenyl]-3-methyl-4-(4-pyrindinyl)benzamide

GR 46611: 2-Propenamide, 3-[3-[2-(dimethylamino)ethyl]-1H-indol-5-yl]-N-[(4-methoxyphenyl)methyl]

GR 55562: 3-[3-(dimethylamino)propyl]-4-hydroxy-*N*-[4-(4-pyridinyl)phenyl]benzamide

GR 65630: 3-(5-methyl-1*H*-imidazol-4-yl)-1-(1-methyl-1*H*-indol-3-yl)-1-propanone

GTI: 5-hydroxytryptamine-5-*O*-carboxymethylglycyltyrosinamide

HTF 919: Hydrazinecarboximidamide, 2-[(5-methoxy-1H-indol-3-yl)methylene]-N-pentyl-, (Z)-2-butenedioate

L 694247: 2-[5-[3-(4-methylsulphonylamino)benzyl-1,2,4-oxadiazol-5-yl]-1*H*-indol-3yl] ethanamine

LY 278584: 1-methyl-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-1*H*-indazole-3-carboxamide

LY 334370: 5-(4-flurobenzoyl)amino-3-(1-methylpiperidin-4-yl)-1*H*-indole fumarate

LY 344864: N-[(3R)-3-(dimethylamino)-2,3,4,9-tetrahydro-1H-carbazol-6-yl]-4-fluoro-benzamide

LY 53857: Ergoline-8-carboxylic acid, 6-methyl-1-(1-methylethyl)-, 2-hydroxy-1-methylpropyl ester, (8b)-, (2Z)-2-butenedioate

mCPP: 2-(2-methyl-4-chlorophenoxy)propanoic acid

MDL 100907: (+/-)2,3-dimethoxyphenyl-1-[2-(4-piperidine)-methanol]

MDL 72832: 8-[4-[[(2,3-dihydro-1,4-benzodioxin-2-yl)methyl]amino]butyl]

MK 212: 4-(6-chloro-2-pyrazinyl)piperazine

MK 462: 1H-Indole-3-ethanamine, N,N-dimethyl-5-(1H-1,2,4-triazol-1-ylmethyl)

ML 10302: 2-(1-piperidinyl)ethyl-4-amino-5-chloro-2-methoxybenzoate

NAD 299: 2H-1-benzopyran-5-carboxamide
PNU 109291: (S)-3,4-dihydro-1-[2-[4-(4-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-methyl-1*H*-2-benzopyran-6-carboximide
Ro 04-6790: 4-amino-N-[2,6-bis(methylamino)-4-pyrimidinyl]- benzenesulfonamide
Ro 600175: (S)-2-(6-chloro-5-fluroindol-1-yl)-1-methyethylamine
Ro 630563: 4-amino-*N*-[2,6-bis(methylamino)pyridin-4-yl]benzenesulphonamide
RS 100235: 1-(8-amino-7-chloro-1,4-benzodioxan-5-yl)-5-((3-(3,4-dimethoxyphenyl)prop-1-yl)piperidin-4-yl)propan-1-one
RS 102221: 8-[5-(5-amino 2,4-dimethoxyphenyl) 5-oxopentyl]-1,3,8-triazaspiro[4,5]decane-2,4-dione
RS 127445: 2-Amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine
RS 23597-190: Benzoic acid, 4-amino-5-chloro-2-methoxy-, 3-(1-piperidinyl)propyl ester, monohydrochloride
RS 39604: Methanesulfonamide, N-[2-[4-[3-[4-amino-5-chloro-2-[(3,5-dimethoxyphenyl)methoxy]phenyl]-3-oxopropyl]-1-piperidinyl]ethyl]-, monohydrochloride
RS 57639: 4-amino-5-chloro-2-methoxy benzoic acid 1-(3-[2,3-dihydrobenzo[1,4]dioxin-6yl)-propyl]-piperidin-4yl methyl ester
RS 57639: 4-amino-5-chloro-2-methoxy benzoic acid 1-(3-[2,3-dihydrobenzo[1,4]dioxin-6yl)-propyl]-piperidin-4yl methyl ester
RS 67506: 1-(4-amino-5-chloro-2-methoxyphenyl)-3-(1-*n*-butyl-4-piperidinyl)-1-propanone
RS 67506: 1-(4-amino-5-chloro-2-methoxyphenyl)-3-(1-*n*-butyl-4-piperidinyl)-1-propanone
RU 24969: 1H-Indole, 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-butanedioate
SB 200646: *N*-(1-methyl-5-indonyl)-*N'*-(3-pyridyl) urea hydrochloride
SB 203186: 1H-Indole-3-carboxylic acid, 2-(1-piperidinyl)ethyl ester
SB 204070: 1-butyl-4-piperidinylmethyl-8-amino-7-chloro-1-4-benzoioxan-5-carboxylate
SB 204741: *N*-(1-methyl-5-indoylyl)-*N'*-(3-methyl-5-isothiazolyl)urea
SB 206553: (5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f]indole)
SB 207710: 1-butyl-4-piperidinylmethyl-8-amino-7-iodo-1,4-benzodioxan-5-carboxylate
SB 216641: [1,1'-Biphenyl]-4-carboxamide, N-[3-[2-(dimethylamino)ethoxy]-4-methoxyphenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)
SB 224289:1'-methyl-5[[2'-methyl-4'-)5-methyl-1,2,4-oxadiazol-3-yl)biphenyl -4-yl]carbonyl-2,3,6,7-tetrahydrospiro[furo[2,3-*f*]indole-3,4'-piperidine]oxalate
SB 236057:1'-ethyl-5-(2'-methyl-4'-(5-methyl-1,3,4-oxadiazol-2-yl)biphenyl-4-carbonyl)-2,3,6,7-tetrahydrospiro[furo[2,3-f]indol3-3,4'-piperidine
SB 242084: 6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbamoyl] indoline
SB 258585: 4-iodo-*N*-[4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-benzenesulphonamide
SB 258719: (R)-3,*N*-dimethyl-*N*-[1-methyl-3-(4-methylpiperidin-1-yl)propyl]benzene sulphonamide
SB 269970: (R)-3-(2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidine-1-sulphonyl)phenol
SB 271046: 5-chloro-*N*-(4-methoxy-3-piperazin-1-yl-phenyl)-3-methyl-2-benzothiophenesulphonamide
SB 272183: 1H-Indole-1-carboxamide, 5-chloro-2,3-dihydro-6-(4-methyl-1-piperazinyl)-N-[4-(4-pyridinyl)-1-naphthalenyl]
SB 357134: *N*-(2,5-dibromo-3-flurophenyl)-4-methoxy-3-piperazin-1-ylbenzenesulphonamide
SDZ 21009: 1H-Indole-2-carboxylic acid, 4-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-, 1-methylethyl ester
SKF 99101H: 1H-Indole-3-ethanamine, 4-chloro-N,N-dimethyl-5-propoxy-, (E)-2-butenedioate
SR 57227: 4-amino-(6-chloro-2-pyridyl)-1-piperidine hydrochloride
U 92016A: 3H-benz[e]indole-2-carbonitrile, 8-(dipropylamino)-6,7,8,9-tetrahydro-, monohydrochloride
UH 301: 1-naphthalenol, 7-(dipropylamino)-4-fluoro-5,6,7,8-tetrahydro-, hydrobromide
WAY 100635: *N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridyl)-cyclohexanecarboxamide trichloride
ZM 170809: 2-Propanamine, N,N,2-trimethyl-1-[(3-phenyl-2-quinolinyl)thio]-monohydrochloride

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ARTICLE

Weak if any effect of estrogen on spatial memory in rats+

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ABSTRACT In a number of species, males appear to have spatial abilities that are superior to those of females. The favored explanation for this cognitive difference is hormonal: higher testosterone levels in males than in females. An alternative explanation focuses on the role of varying levels of estrogens in females during the estrus cycle; females perform as well as males on days of low estrogen, but more poorly on days of high estrogen. Other investigators have reported that estrogens improve both types of memory processes, which depend on the striatal (nonspatial navigation) and hippocampal (spatial) memory systems. Additionally, estrogens have been found to protect the working memory. These contradictory results initiated the present study, in which ovariectomized female rats were trained to escape in a Morris water maze. The daily trials were preceded by estradiol application in low doses (Experiment I) or in higher doses (Experiment II). In Experiment I, no differences at all were found between the latencies of the treated and control groups to reach a submerged platform in a Morris water maze. In Experiment II, however, the animals treated with the higher dose of estradiol showed a small deficit in the acquisition of the Morris water maze task. This study indicates that estradiol at around the physiological level has no effect on spatial learning and memory functions.
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KEY WORDS

estrogen
Morris water maze
spatial learning
memory functions

One of the clinical symptoms reported by menopausal and postmenopausal women is a deficit in memory and cognitive functions (Kopera 1973; Brown 1976). There has recently been mounting evidence that estrogen receptors (ER) are also involved in "nonreproductive" behaviour, including that associated with the hippocampal spatial discrimination function (McEwen et al. 1997). For example, during proestrus, or following estradiol injections, female rats exhibit a poorer performance in the spatial version of the water escape task, which is sensitive to the hippocampal function (Korol et al. 1994; Berger-Sweeney et al. 1995; Frye 1995). Furthermore, the hippocampal anatomy and physiology are altered by these estradiol level or by estrogen treatment, suggesting possible neurobiological substrates for estrogenic effects on spatial performance and memory (Warren et al. 1995).

There are several putative mechanisms through which estrogens might affect memory, including the following: 1) the potential of estradiol to alter the glutamate sensitivity of the hippocampal neurons (Weiland 1992a); 2) the estradiol-induced activation of a subset of hippocampal GABA neurons (Weiland 1992b); 3) the putative action of estradiol on choline acetyltransferase (Luine et al. 1980); 4) the ability of estradiol to increase cyclic adenosine monophosphate (cAMP) levels in the hypothalamus (Gunaga et al. 1974); and 5) the effects of estrogens on neuronal plasticity, which has been well documented in several regions of the central nervous system (Chung et al. 1988; Párducz et al. 1993; Langub et al. 1994; Holstege 1997; VanderHols and Holstege 1997; Wooley 1998; Horváth et al. 2002).

As indicated above, the effects of estrogens in "nonreproductive" behaviour are well documented. However, the effects of estrogens on the learning and memory functions are rather controversial. Some researchers observed no effects of estrogens on the learning and memory functions (Healy et al. 1999; Wilson et al. 1999), whereas others reported a negative effect (Fuger et al. 1998; Chesler and Jaruska 2000), and some laboratories found estrogens to exert enhancing action in spatial memory tasks (Rissanen et al. 1999; Gibbs 2000).

In order to determine whether estrogen pretreatment has any effect on a spatial learning task, the present study was carried out in a Morris water maze.

Materials and Methods

Subjects and surgery

A total of 40 adult Sprague-Dawley rats were raised with access to water and food pellets (Altromin) *ad libitum*.

At the age of 3 months, all the animals were ovariectomized (OVX) by means of bilateral dorsal incisions. All the surgical procedures were carried out under deep Ketamine/xylazine anaesthesia (Ketamine 10.0 mg/100 g and xylazine 0.8 mg/100 g body weight, i.p.).

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+Dedicated to Professor Otto Fehér on the occasion of his 75th birthday.

Hormone administration

Experiment I: Fourteen days after ovariectomy, 22 rats were divided into two groups: 1) those (12) that received a single dose of 17β-estradiol (20 μg/kg in sesame oil, i.p.) and 2) those (10) that received an injection of vehicle alone. The first injection was given 28 h before the experiments, and the subsequent injections 4 h before the first trial of each day.

Experiment II: Eighteen rats were divided into two groups (10 and 8, respectively), and an experiment similar to that described above was carried out, except that the dosage of estradiol was higher (100 μg/kg).

Behaviour

Rats were tested with the spatial version of the Morris swim task, starting 2 weeks following gonadectomy. The animals were trained in a circular pool (160 cm in diameter) located in an artificially-lighted room containing an assortment of two- and three-dimensional cues (posters, lamp, etc.). The pool water (40 cm deep) was maintained at room temperature (22 ± 0.5°C) and a white escape platform (10 cm in diameter) was situated approximately 1.5 cm beneath the water level. The escape platform was positioned at the center of one of the four quadrants of the pool and remained in the same location throughout testing.

In order to acclimatize the rats to the task, they were first placed into the water for a 30-min "free swim" and then assisted onto the platform from three different directions. A 30-s rest on the platform was then permitted before the first training trial was administered. The training consisted of three blocks of four trials per day for 4 consecutive days. Intertrial intervals were 20 s in duration while interblock intervals lasted approximately 15 min. In each trial, the rat was placed in the water from one or other of the four equally spaced start locations (N, S, E and W). A period of 60 s was available for the rat to escape during each trial. If it did not escape within that time, it was gently guided to the platform, and a time of 60 s was allocated as the escape latency. Between trials, the rats remained on the platform. After each block and between blocks, the rats remained in their home cages.

Analyses

To assess the acquisition of the spatial task, latency measures (the time to reach the platform) were composed across blocks of trials, using repeated measures analysis of variance (ANOVA).

Results

Experiment I

Figure 1 illustrates the mean latencies of the Morris water task for the rats treated with 20 μg/kg estradiol (n=12) or oil (n=10). A decrease in escape latency over the course of training is routinely used as an indicator that the animals have acquired a successful strategy of escaping from the pool. As Figure 1 shows, both groups acquired the successful strategy, and the analysis did not reveal any significant difference in performance between them.

Experiment II

In this experiment, the animals treated with 100 μg/kg estradiol also acquired the strategy of escaping from the pool. On the first day, the performances of the animals in the two groups were very similar, the difference between them not being significant. During the following days, the animals treated with estradiol did not show such good progress in acquiring the spatial discrimination test as did the control group. However, the analysis indicated a slight but significant difference between the two groups only on the third day (Fig. 2).

Discussion

Since the literature on the effects of estrogens on spatial learning and memory functions is rather controversial, we carried out two series of experiments, in which the effects of two different doses of estradiol were compared.

In Experiment I, we used a dose of estradiol comparable to the blood level found in mice (Rissanen et al. 1999) and to the dose administered to rats in behavioural studies (Vongher and Frye 1999; Chesler and Juraska 2000). The higher dose in Experiment II is comparable to that applied by others (Fugger et al. 1998; Isgor and Sengelaub 1998) or by ourselves in an electrophysiological study (Kis et al. 1998).

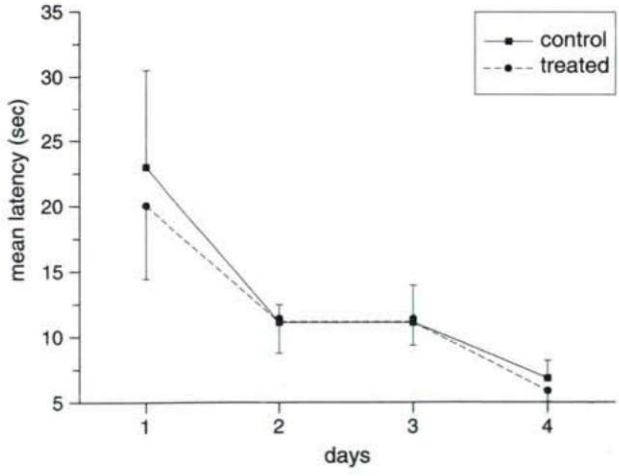

Figure 1. Experiment I: Maze performances of controls and animals treated with 20 μg/kg estradiol. As the results show, both groups acquired the successful strategy to escape. The analysis did not reveal any difference in performance between them. Bars represent mean (± SEM) latencies.

In the present study, at the low dosage (comparable to the physiological level), we found no difference between the performances of the treated and control groups.

Previous researches suggested that, during the breeding season (high estradiol), female voles show poor performance as compared to males in the spatial version of the water escape task (Galea et al. 1995). Our result is in accord with that observation; after the high-dosage estradiol treatment, the animals displayed a somewhat poorer performance in the water maze task than did the controls. These results are consistent with the findings in some other studies (Fugger et al. 1998), but seem to contradict those which found an improved performance related to estradiol treatment (Packard and Teather 1997a, b).

The reason for this discrepancy might be that, in addition to an impaired acquisition following acute estradiol treatment, when given over longer time periods, this estrogen can act through other mechanisms to improve memory functions. In fact, an improved memory can be observed in gonadectomized rats following chronic estrogen treatment (O'Neal et al. 1996).

Though it is possible that chronic estrogen treatment mediates an additional set of neuronal modifications, which in turn facilitate memory, our present study shows that acute estradiol treatment with a dosage within the physiological range has no effect on the spatial learning and memory functions. Treatment with a higher dosage of estradiol tends to impair the performance of the treated animals.

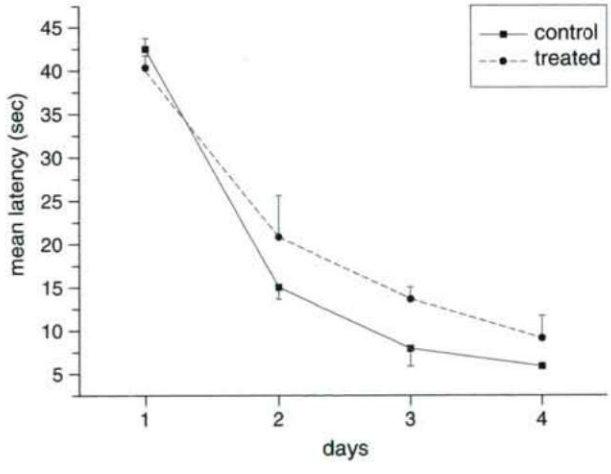

Figure 2. Experiment II: Maze performances of controls and animals treated with 100 µg/kg estradiol. The results demonstrate a small deficit in the acquisition of the Morris water maze task; however, the difference between them was significant only on the third day (t=2,3, p=0.0355). Bars represent mean (± SEM) latencies.

Acknowledgments

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ARTICLE

Individual distribution and colocalization of nitric oxide synthase with vasoactive intestinal polypeptide and neuropeptide Y in the developing human fetal small intestine

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ABSTRACT The appearance, the individual distribution and the pattern of colocalization of nitric oxide synthase (NOS-immunoreactivity, NOSi), vasoactive intestinal peptide (VIP-immunoreactivity, VIPi) and neuropeptide Y (NPY-immunoreactivity, NPYi) immunoreactivity were examined in the developing human fetal small intestine at weeks 12 and 18 of gestation. Neurons expressing VIPi, NPYi and NOSi were observed in the small intestine of the 12-week-old human fetuses and from this age on a gradual increase in the immunoreactivities appeared until week 18 of gestation when a dense network of immunopositive fibres and cell bodies were observed both in the submucous (SmP) and in the myenteric plexuses (MP). The double-labelling immunocytochemistry showed different pattern of the overlapping immunoreactive structures within the myenteric and submucous plexuses. The cellular colocalization of VIPi and NOSi in submucous ganglia were revealed around week 12 of gestation while in the myenteric ganglia cells with overlapping immunoreactivity appeared around week 18. A limited cellular colocalization of NPYi and NOSi were noticed before week 18, and NOSi neurons in the MP of the 12-week-old fetuses were preferentially innervated by NPYi varicosities. These results suggest that VIP, NPY and NO may exert a cooperative action in human fetal gastrointestinal motility.

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KEY WORDS

human intestine
immunocytochemistry
NOS
VIP
NPY

Detailed studies about the ontogeny of peptide-containing neurons in the human enteric nervous system (ENS) were performed (Bryant et al. 1982; Larsson et al. 1987) and it is known that neuronal elements expressing VIP or NPY appear during early fetal development. Many studies have suggested that VIP containing neurons are important mediators of the descending inhibitory phase of peristalsis (Larsson et al. 1976; Hata et al.1990; Furness et al. 1992; Grider and Jin 1993; Allesher and Daniel 1994). Other evidence indicates that the mediation of descending inhibition is not limited to VIP and suggests that nitric oxide (NO) may also serve as an inhibitory neurotransmitter (Giorgio et al. 1994; Yuan et al. 1995; Young et al. 1995). Recent evidence indicates that NPY is present in inhibitory motor neurons of guinea pig mye/nteric ganglia (Uemura et al. 1995). The wide distribution of neurons with NPYi suggests that just like VIP, NPY is also involved as an inhibitory neurotransmitter in all regions of the fetal gut. There are two populations of NPY immunoreactive nerve fibres in the gut. One major population of fibres is of intrinsic origin, distributed in all layers of the gut (Sundler et al. 1993). A minor population of NPY fibres, with extrinsic origin, is identical with the adrenergic NPY fibres distributed mainly around blood vessels and in the myenteric ganglia (Lundberg et al. 1982; Browning et al. 1999). Colocalization studies showing that NO is produced in enteric neurons that express neuropeptides including VIP (Costa et al. 1992) and NPY (Kirchgessner et al. 1994) make VIP, NPY and NO viable candidates as parallel neurotransmitters.

The early appearance of neuronal elements with VIP, NPY and NOS immunoreactivity is well documented in human fetal intestine (Bryant et al. 1982; Chayvialle et al. 1983; Larsson et al. 1987; Timmermans et al. 1994). Since most of these investigations were performed on sections, they do not give informations about the distribution of nerve fibres and cell bodies within the different compartments of the human fetal ENS. Studies as concerns the possible co-localization of VIPi with NOSi or NPYi with NOSi during the development of the human ENS do not exist. The first aim of these investigations was therefore to determine the individual distribution of VIPi, NPYi and NOSi within the human fetal ENS using wholemount preparations of the intestinal wall. The second aim was to investigate in what extent these

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substances are colocalized in the developing human fetal small intestine. The third aim was to examine changes in the distribution of the overlapping immunoreactive neuronal elements between weeks 12 and 18 of gestation.

The present study provides the first evidence on the simultaneous appearance of NOSi with VIPi and NOSi with NPYi in different neuronal populations in the developing human fetal small intestine.

Materials and Methods

Tissues

Intestinal segments of human fetuses (weeks 12 and 18 of gestation) were obtained immediately after legally approved or spontaneous abortions. The crown-heel length was used to assign gestation age. Three fetuses of all ages were used for each examination. The experiments were performed in accordance with the declaration of the Medical World Federation proclaimed in Helsinki in 1964.

Immunocytochemistry

Segments of small intestine were ligated and distended using a modified Zamboni fixative (Scheuermann et al. 1987) and fixed overnight at 4°C. After washing with phosphate buffered saline (PBS) at pH 7.4, tissue pieces were used for wholemount preparations and cryosections. Double-labelling immunofluorescence histochemistry was performed applying simultaneous incubation using a monoclonal mouse anti-NOS antiserum (Affinity, Menasha, USA; final dilution 1:200) in combination with either a rabbit anti-VIP (Affinity; final dilution 1:200) or a rabbit anti-NPY antiserum (Amersham; final dilution 1:500) overnight at room temperature. After incubation with primary antisera wholemounts were washed with PBS and exposed for 6 h to a mixture of species-specific secondary antibodies conjugated to FITC (Jackson, Baltimore, USA; final dilution 1:100), Cy3 (Sigma, Budapest, Hungary; final dilution 1:200) or biotin (Amersham, Backinghamshire, England; final dilution 1:100). After incubation in secondary antiserum, tissues were washed and incubated for overnight with streptavidin-Texas Red (Amersham; final dilution 1:100) or streptavidin-biotinylated horseradish peroxidase (Amersham; final dilution 1:100). Specimens were mounted in PBS-buffered glycerol. Preparations were viewed and photographed with a Zeiss Axioscope 2 MOT fluorescent microscope equipped with a Zeiss AxioCam digital camera.

Results

The individual distribution and the pattern of coexistence of NOSi, VIPi and NPYi were examined in cryosections and in wholemount preparations of human fetal small intestine between weeks 12 and 18 of gestation. NOS, VIP and NPY immunoreactive structures were observed in each part of the small intestine at week 12 of gestation (Figs. 1 and 2). From this age on there was a gradual increase in the intensity of immunofluorescence, and also in the number and the diversity of the immunopositive nerve structures until week 18 of gestation, when dense networks of immunopositive fibres and large number of immunopositive cell bodies were seen (Fig. 3). Most of the peptide-containing intraganglionic fibres expressing either VIP or NPY were distributed within the myenteric plexus (MP) as varicose fibres and freqently formed baskets around non-immunopositive cell bodies (Figs. 1D, F and 2D, 2F). NOSi fibres within the MP showed a dense, less structured pattern of fibres with rare varicosities which never formed baskets around non-immunopositive cell bodies (Fig. 2A). Both peptidergic and nitrergic neuronal structures were less densely distributed in the submucosus plexus (SmP), although varicose fibres and cell bodies with VIPi are frequently seen at 12 week of gestation (Figs. 1C and E). NOS was also expressed in the submucous fibres from week 12 on (Fig. 1B). Double-labelling experiments revealed a limited coexistence of VIPi with NOSi and NPYi with NOSi depending on the embryonic age examined. NOSi with VIPi or NOSi with NPYi were never expressed together in the varicosities of the embryonic ENS. The dense varicosities of peptidergic fibres expressed only VIP or NPY, whereas the scarce varicosities containing NOS never expressed peptides. Three populations of immunoreactive cell bodies were revealed after double-labelling with VIP and NOS: a population of myenteric neurons co-expressing VIP with NOS (Figs. 2C and D) and another two populations containing VIP or NOS alone (Figs. 2C, D and Figs. 3E, F). A population of nerve cell bodies in submucous ganglia were observed that express VIPi and NOSi together from week 12 of gestation. Nerve cells after double-labelling with NPY and NOS also fall into three classes: a population containing NPY with NOS (Figs. 2A and B) and another two populations expressing NPY or NOS alone (Figs. 2E and F). The number of cell bodies expressing NPY alone or NPY and NOS together were limited to one or two cells per ganglia.

Discussion

Our present investigations covered the localization of three substances: NO, VIP and NPY, known to occur in neuronal elements of human adult intestine. VIP-containing nerve fibres occur abundantly in all layers of the human gut and VIP nerve cell bodies are regularly observed in intramural ganglia (Bishop et al. 1982). The presence of the regulatory peptides and NO has been proved in the early human fetal intestine (Bryant et al. 1982; Timmermans et al. 1994). Most of the peptides studied throughout the fetal development had an adult-like distribution pattern by weeks 20 and 24 of gestation (Bloom et al. 1983). As well as having a regulatory role in intestinal motility, relaxing smooth muscle and mediating inhibitory non-adrenergic non-cholinergic

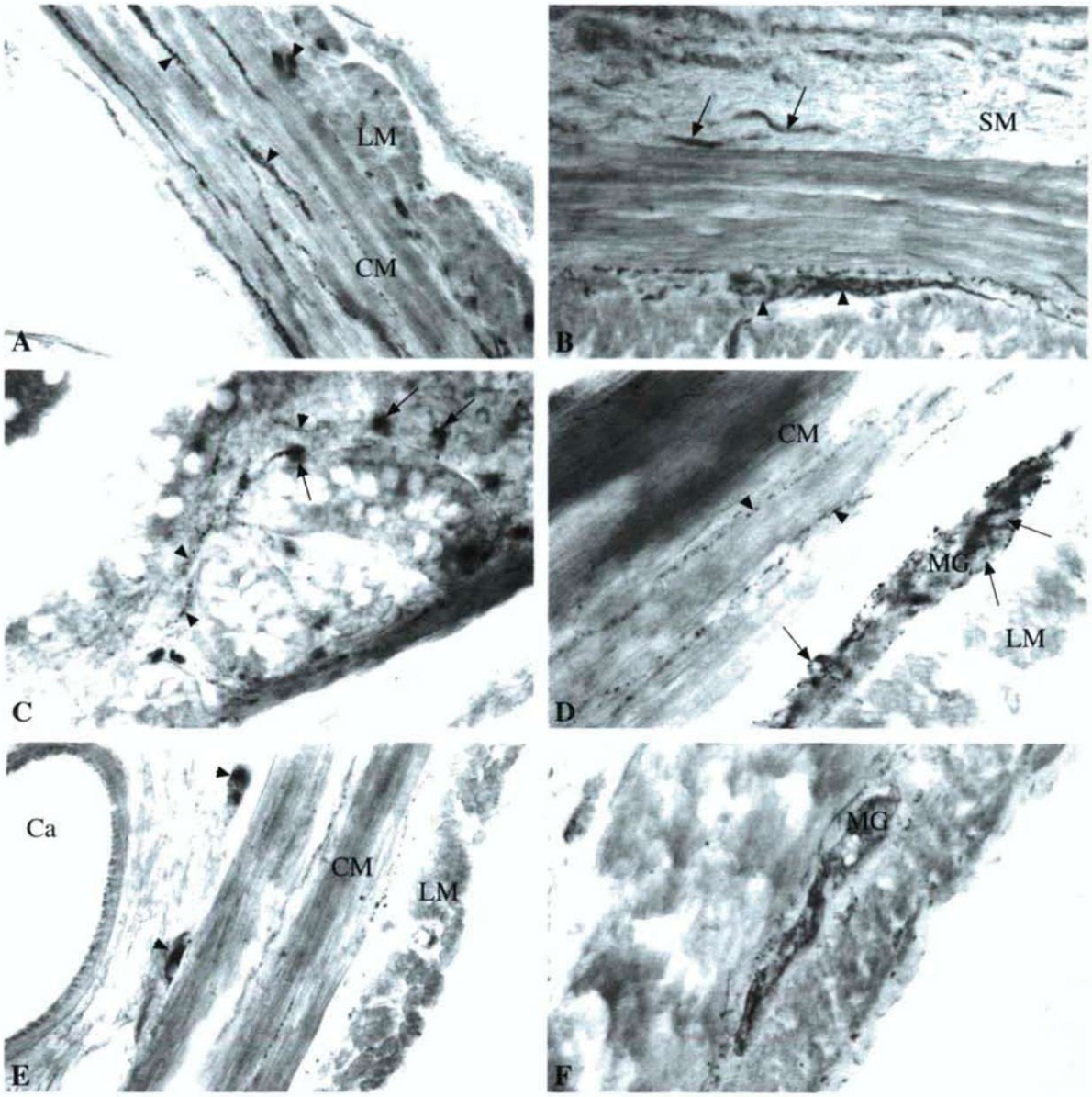

Figure 1. Light photomicrographs of cross-sections of the human fetal gut wall after NOS (A, B), VIP (C, D, E) and NPY (F) immunocytochemistry on week 12 of gestation.

A. NOS-immunopositive neuronal elements (arrowheads) are abundant both in the longitudinal (LM) and circular muscle (CM) layers. x200

B. NOS-immunopositive neuronal elements are present in myenteric ganglia (arrowheads). Smooth individual fibres in the submucous layer (SM) with NOS-immunopositivity appear (arrows). x400

C. VIP-immunoreactive varicose fibres (arrowheads) and cell bodies (arrows) around glandular epithelia in the submucosal layer on week 12 of gestation. x650

D. VIP-immunoreactive varicose fibres form baskets around non-immunoreactive cell bodies (arrows) within a myenteric ganglion. Dense array of VIP-immunoreactive fibres (arroheads) was also revealed in the circular muscle layer (CM). LM: longitudinal muscle layer, MG: myenteric ganglion. x400

E. VIP-immunoreactive cell bodies (arrowheads) in the submucous plexus. CM: circular muscle layer, LM: longitudinal muscle layer, Ca: capillary, x200

F. NPY-immunopositive neuronal elements in a myenteric ganglion (MG). x400

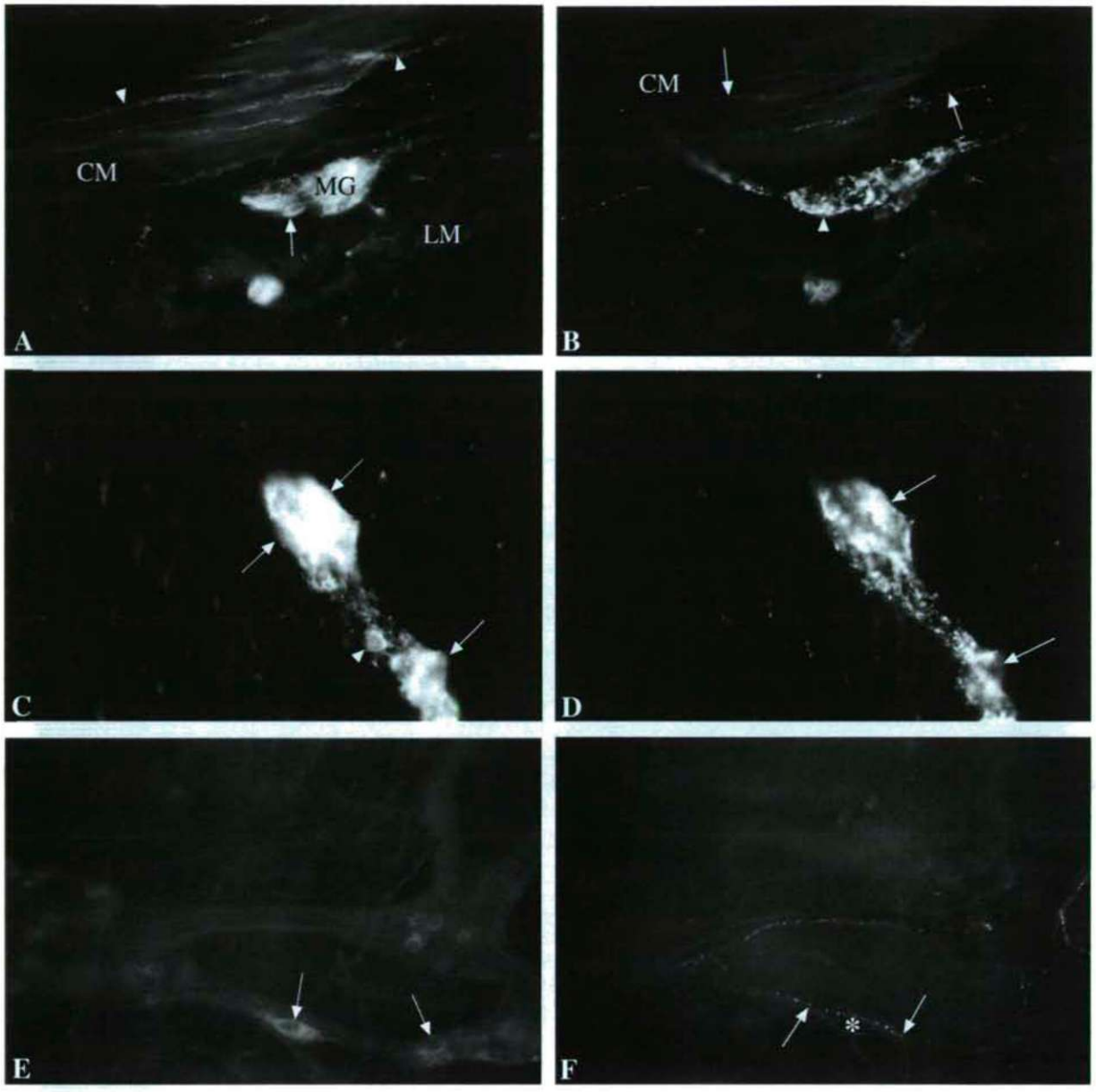

Figure 2. Fluorescent micrographs of cross-sections (A-D) and wholemounts of the human fetal gut wall after double-labelling immunocytochemistry for NOS, NPY (A, B, E, F) and NOS, VIP (C, D) on week 12 of gestation.
A. NOS-immunopositive neurons (arrow) in a myenteric ganglion (MG) and dense arrays of fluorescent fibres (arrowheads) in the musculature. CM: circular muscle layer, LM: longitudinal muscle layer, x400
B. NPY-immunoreactive varicose fibres (arrows) in the circular muscle layer (CM) and in a myenteric ganglion. Some of the ganglion cells coexpress NOS and NPY (arrowhead). The NPY-positive fibres form baskets around non-immunreactive myenteric neurons. x400
C. NOS-immunopositive neuronal elements in a myenteric ganglion. Most of the cells express NOS and VIP together (arrows), while others express only NOS. x650
D. VIP-immunopositive neuronal elements in a myenteric ganglion. Most of the ganglion cells co-express NOS and VIP (arrows). x650
E. NOS-positive neurons in the myenteric plexus (arrows). x200
F. NPY-positive varicose fibres in the myenteric plexus. A nitrergic neuron (asterisk) is surrounded by NPY immunopositive fibres (arrows). 200x

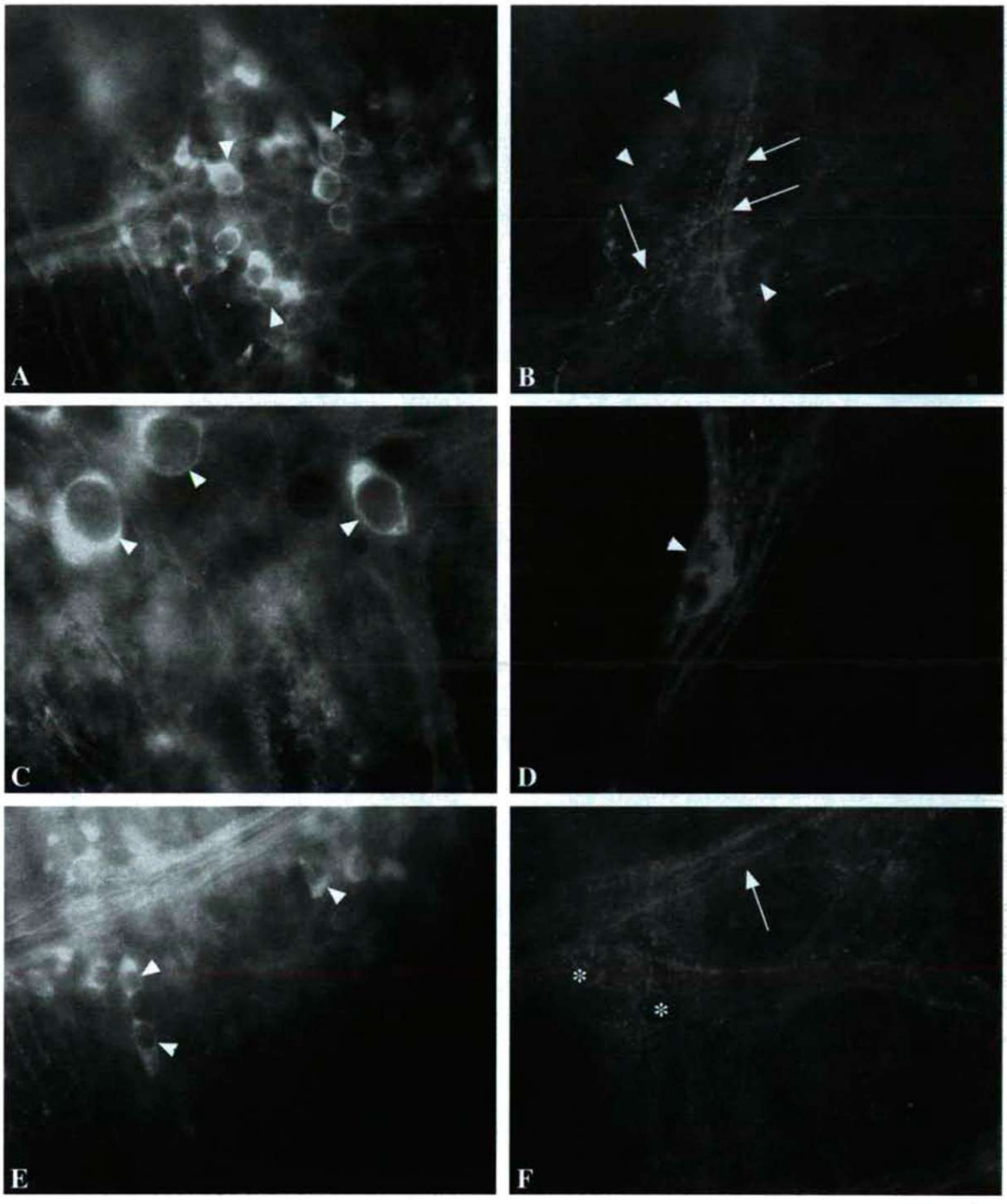

Figure 3. Fluorescent micrographs of wholemount preparations of the human fetal small intestine after single labelling immunocytochemistry for NOS (A, C), VIP (B, D), and double labelling immunocytochemistry for NOS, VIP (E, F) on week 18 of gestation.
A. NOS-immunopositive neurons (arrowheads) in a myenteric ganglion. x400
B. Dense array of VIP-immunopositive varicose fibres (arrows) and cell bodies (arrowheads). x400
C. NOS-immunopositive neuronal cell bodies (arrowheads) in a myenteric ganglion. x1000
D. VIP-immunopositive neuronal cell body (arrowhead) in a myenteric ganglion. The immunoreactivity is unevenly distributed within the perikaryon. x1000
E. NOS-immunopositive neuronal cell bodies (arrowheads) in a myenteric ganglion. x400
F. Dense array of the VIP-immunopositive varicose fibres (arrow) in the myenteric plexus. Varicose fibres frequently form baskets (asterisks) around non-immunoreactive cell bodies. x400

(NANC) neurotransmission (Larsson et al.1976; Furness et al. 1992; Grider and Jin 1993; Allesher and Daniel 1994), the ability to act as growth factors has also been demonstrated both for NO (Ogura et al. 1996) and VIP (Gressens et al. 1993). In order to evaluate the potential interactions of NO, VIP and NPY in the developing human fetal small intestine double-labelling immunocytochemistry was used and simultaneous appearance of NOSi with VIPi and NPYi with NOSi in the different neuronal structures was followed from week 12 of gestation, when immunoreactive neuronal elements were widely distributed in the fetal ENS. While both VIP and NPY were localized mainly to varicose fibres and they frequently formed baskets around cell bodies, the majority of fibres with NOSi were smooth and never formed baskets around cell bodies. The number of cells expressing NOS was higher in the MP than those expressing VIP, while in submucous ganglia the number of VIPi neuronal cell bodies overwhelmed the one or two NOSi cells per ganglia. Due to the high number of cells expressing VIP it can be assumed that most of the fibres displaying VIPi are intrinsic of the fetal gut. Both the peptidergic and the nitrergic neuronal structures were less densely distributed in the SmP. However, varicosities within the SmP with VIPi were not seen before week 18 of gestation, cells expressing VIP and NOS alone or together appeared already at week 12. On the contrary, cellular colocalization of NOSi and NPYi was not revealed within the SmP; however, the scarce NOS immunreactive neurons frequently received NPY immunreactive fiber terminations suggesting a modulatory role for NPY (Cox et al. 1998; Feletou et al. 1998).

The coexistence of both VIPi with NOSi and NPY with NOSi was most pronounced in the 18-week-old fetus, when the pattern of colocalization is similar using either VIP or NPY antibody in combination with NOS antibody. The neurons which simultaneously expressed either VIP and NOS or NPY and NOS fall into five groups, two populations contained NOS and VIP or NOS and NPY together, another populations contained NOS, VIP or NPY alone. The NOS immunreactive neurons which have a dominant NPY innervation might represent a subgroup of NOS neurons. The physiological significance of this pattern of coexistence is still a subject of debates. Due to the early expression of these substances (Bryant et al. 1982; Chayvialle et al. 1983; Larsson et al. 1987; Timmermans et al. 1994), they might have their individual action during the early development, relaxing smooth muscle (Costa et al. 1992; Grider and Jin 1993; Kirchgessner et al. 1994) and acting in NANC neurotransmission. Later in development, when oro-anal peristalsis starts (Grand et al. 1976) and the motility of the gut needs a more sophisticated regulation, a complementary role might be presumed to these substances. NO may mediate the same type of response as VIP or NPY but with a different time course. The different classes of neurons suggest that in some cases NO is the final transmitter but most of the cases it probably serves as a modulator to another NANC neurotransmitter or it has an effect on the regulation of development. The lack of varicosities in submucous fibres expressing VIP or NOS suggests that NANC inhibition is not an important part of the submucous function in the early development of human ENS. The early appearance of cellular colocalization at the same time suggests a regulatory effect of these substances either in neurotransmission or in neuronal differentiation.

In conclusion, the distribution and the pattern of coexistence revealed in our present investigation using double-labelling experiments with NOS and VIP or NOS and NPY antibodies, strongly support the concept that VIP with NOS and NPY with NOS are parallel neurotransmitters in the human fetal small intestine, although the neurotrophic effect of NO and VIP should be a subject of further investigation.

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DISSERTATION SUMMARY

Modification of bacterial enzymes using in vitro evolution methods

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The aim of our project is the investigation of the *in vitro* evolutionary adaptation of bacterial enzymes. The molecular basis of the heat stability of enzymes is poorly understood. The amino acid sequences and the three-dimensional structures of thermophilic enzymes and their mesophilic counterparts are usually similar. In order to change the heat-stability, many laboratories applied directed mutagenesis techniques, based on the known amino acid sequences of the homologous enzyme pairs, but the results of these experiments were not always predictable. Now we know that each thermophilic protein is stabilised by unique combinations of molecular interaction (salt bridges, hydrophobic interactions, stabilization of loops, etc.)

That is why we decided to use *in vitro* methods that mimic evolution. We chose one homologuous enzyme pair, namely HisF, from the mesophilic eubacterium *Escherichia coli* and the thermophilic archaeon *Methanococcus jannaschii*. In our experiments we randomly mutagenized the thermophilic *Methanococcus hisF* gene using error-prone PCR and a mutator strain to convert the enzyme coded by this gene into a mesophilic enzyme. We constructed recombinant plasmids, which carry the *hisF* gene of either *M. jannaschii* or *E. coli* inserted in the vector plasmid pBAD24. In these constructs the expression of the *hisF* genes are under the control of a promoter that can be regulated by the concentration of arabinose in the growth medium. The mutagenized and wild type plasmids were transformed into an *E. coli hisF*$^-$ mutant host and the transformants were grown on medium containing the inducer arabinose. The *E. coli hisF*$^-$ mutant cells expressing *M. jannaschii* HisF grew much slower than those expressing *E. coli* HisF. We have isolated a colony carrying a mutant *Methanococcus* gene variant which grew faster at 37°C than those bacteria expressing the wild type thermophilic enzyme. In this gene we detected a missense mutation near the catalytic site. Thereafter we intended to characterize this mutant enzyme. To measure HisF activity we had to synthetize and purify its substrate, and set up the appropriate assay conditions.Having done this, currently we are in the process of determining the temperature optimum and heat stability of the mutant HisF enzyme.

The other part of our work was to engineer enzyme variants with novel catalytic properties. The *SinI* modification methyltransferase *(M.SinI)* methylates the sequence GGA/TCC. We wanted to convert the substrate specificity of *M.SinI* into the more relaxed specificity GGNCC. Our approach was to use random mutagenesis and *in vitro* DNA shuffling to isolate variants of *M.SinI* that have lost the capacity to discriminate between A/T and G/C base pairs in the center of the recognition sequence. A heterogeneous plasmid pool carrying the mutagenized and shuffled M.*Sin*I gene was digested with *Sau96*I endonuclease, an enzyme that recognizes the DNA sequence GGNCC. The selection was based upon the idea that the required M.*Sin*I mutants which have lost the ability to recognize the central A/T base pair would methylate all *Sau96*I (*i.e.* GGG/CCC and GGA/TCC) sites in the plasmid, making it resistant to *Sau96*I digestion. Recently, we successfully isolated such a *Sau96I* resistant clone. Sequencing this clone, characterization of the enzyme coded by this mutant gene, quantitative assessment of its methylation specificity is currently in progress.

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DISSERTATION SUMMARY

Characterization of a putative respiratory complex in the hyperthermophilic archaeon, *Thermococcus litoralis*

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Thermococcus litoralis, isolated from a shallow submarine thermal spring in Italy, is a hyperthermophilic archaeon. It is an obligate heterotroph, growing at temperatures between 55 °C and 98 °C, with an optimum around 85 °C, and it is able to reduce sulfur.

While isolating the genes of the soluble hydrogenase I of *T. litoralis*, we found open reading frames in the upstream region, in the opposite transcriptional direction to the hydrogenase genes. So far, a region of approximately 10 kb was isolated from a partial genomic library and sequenced. In this region, 8 orfs, namely *leg1-8* were found. Their genomic organization – overlap of the start and stop codons with the subsequent orfs, or the existence of just 2-3 bp long gaps – suggests that they form an operon.

Upstream from the first orf, a typical archaeal promoter can be assigned, containing BoxA and BoxB conserved regions. Typical ribosomal binding sites precede all of the open reading frames. Downstream from the *leg8* orf, there is a possible hairpin structure forming sequence motif, which can be a transcriptional termination signal. Attempts are being made to determine the length of the transcript(s) by Northern analysis.

Based on the deduced amino acid sequence, prediction programs suggest that proteins coded by *leg 1,2,6,7,8* are soluble, while *leg 3,4,5* code for transmembrane proteins.

Comparing these sequences to a protein sequence database, we cannot precisely determine the function of the proteins coded by these putative genes. All proteins, found to be similar in the database comparisons, are the members of different energy conserving respiratory systems, such as the NADH:ubiquinone oxidoreductase complex or the formate hydrogen lyase system of *E. coli*.

The aim of the project is to determine the physiological role of this putative enzyme complex. The lack of a recombinant genetic system for these organisms makes impossible to produce and analyze mutants. Without knowing the function of the proteins, mutagenesis with chemical agents is also impossible, because we are unable to screen the mutant library. A possible solution is the complete or partial isolation of the putative protein complex to determine its activity.

In order to follow the isolation of the complex, we decided to raise antibodies against the LEG2, LEG3 and LEG8 proteins. LEG2 and LEG8 were overexpressed in *E. coli* using the pET protein expression system. The His-tag containing recombinant proteins were purified by metal affinity chromatography.

All of our attempts to overexpress LEG3 failed. LEG3 appears to be an integral membrane protein, thus its hydrophobicity likely caused the difficulties in overexpression. To overcome this problem, a 10 amino acid stretch of the C-terminal end of LEG3 was chosen with the help of antigenic region determining programs. This 10mer peptide was custom-synthesized and conjugated to KLH.

With these antibodies, we will be able to follow the purification steps and, with Western hybridization experiments, we can determine the cellular location of these proteins and probable expression regulation can be studied under different growth conditions. The antibodies can also be used in immunoelectrophoresis, which makes possible to link the activity (stained in native gel) to the product of the leg orfs with the use of antibodies.

In parallel with the antibody production, we are making efforts to detect enzyme activities in the membrane fraction of *T. litoralis*, which can be inhibited with well known respiratory chain inhibitors, such as piericidin A.

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DISSERTATION SUMMARY

Studies on hydrogen metabolism of hyperthermophilic *Thermococcus litoralis*

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Thermococcus litoralis is a hyperthermophilic archaeon growing optimally at 85°C. In contrast with the majority of *Thermococcales* species, which are obligately dependent on peptides and sulfur, *T. litoralis* is able to utilize both peptides and carbohydrates as sole carbon and energy source. Sulfur is not necessary for its growth, but has positive effect on the cell yield, likely due to a bioenergetics aspect. The primary end-products of the fermentative metabolism of *T. litoralis* are acetate, CO_2 and H_2 (or H_2S). The cells remove the excess electrons formed during fermentative metabolism via H_2 with the aim of hydrogenases. However, in the presence of S°, H_2S is formed instead of H_2. The hydrogenases seem to participate in the sulfur reduction as well.

So far, a cytoplasmic, heterotetrameric [NiFe] hydrogenase (Hyh1) has been characterized in *T. litoralis*. Recently, in a related strain, *P. furiosus*, two other [NiFe] hydrogenases were identified, and their genes were fished out from its genome. In *T. litoralis*, we have also found the genes coding for proteins corresponding to the γ-subunit of soluble hydrogenase II (Hyh2) and the α-subunit of H_2-evolving, membrane-bound hydrogenase complex (Mbh) described in *P. furiosus*.

Our aim was to clarify the physiological role of the [NiFe] hydrogenases in *T. litoralis*. The analysis of *T. litoralis* hydrogenase mutant strains would have allowed us to determine the function of the hydrogenases. However, there were no usable genetic tools for these microorganisms.

So, we intended to develop a genetic system based on antibiotic selection for *Thermococcales* cells. We have found, that puromycin was effective against *T. litoralis* cells. The lethal concentration of this antibiotic was about 15 μg/ml. In other microbes the resistance of cells against puromycin was provided by puromycin N-acetyltransferase (Pac), which was a moderately thermostable enzyme. A self-replicating vector construction was prepared, which contained the *pac* gene between the promoter and terminator region of the glutamate dehydrogenase gene of *P. furiosus* and a replication origin of a plasmid isolated from a *Pyrococcus* species. Many different transformation strategies were attempted to introduce the vector to the cells, for example, chemically-induced transformation, electroporation, transformation with liposomes, but so far these attempts were unsuccessful. However, there are other alternative protocols, which will be used in the near future.

Another approach to determine the function of the hydrogenases is the investigation of their biosynthesis in the function of the different type of fermentative metabolism of the cells. The regulation of the expression of the hydrogenases has been studied in cells growing on various carbon sources in the presence or absence of S°.

T. litoralis cells have been cultured in well-defined media, which were occasionally supplemented with maltose and/or enzymatically-hydrolyzed casein and/or S°. The effect of these various conditions on the hydrogenases were examined at the level of transcription, translation and the active enzymes.

The specific H_2-evolution activity of whole cells depended on the growth phase of culture. In the presence of S°, the H_2-evolution activity was increased, if maltose or peptides were included in the medium. However, the S° decreased the specific H_2-evolution activity of *T. litoralis* cells grown in defined medium without maltose or peptides. Western blot analysis revealed significantly less amount of Hyh1 in cells grown in the presence of sulfur. Furthermore, the relative amount of Hyh1 proteins was notably higher in *T. litoralis* cells grown without peptides or carbohydrates, than with these supplements. Similar conclusion could be obtained from preliminary real-time RT-PCR experiments, where expression of the Hyh1 was measured at transcription level. Further RT-PCR studies are being performed to confirm these data, and characterize the environmental factors and the multi-sided transcriptional regulatory mechanism controlling the activities of the various hydrogenases. This might lead to understand the physiological roles of each enzyme in more details.

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DISSERTATION SUMMARY

Investigation of the copper-regulated expression of methane monooxygenases in *Methylococcus capsulatus* (Bath)

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Methanotrphic bacteria or methanotrophs are Gram negative aerob bacteria. They are unique in their ability to utilise methane as a sole energy and carbon source. Methane is the most stable carbon compound in anaerobic environments and is a very important intermediate in the reaction that eventually led to the mineralization of organic matter. Methane escapes from the anaerobic environments to the atmosphere when it is not oxidised by methanotrophs. The release of the methane to the atmosphere results in an increased rate of global warming and causes other changes in the chemical composition of the atmosphere.

The use of enzymes known as methane monooxygenases to catalyse the oxidation of methane to methanol is a defining characteristic of methanotrophs. The methanotroph *Methylococcus capsulatus* strain Bath (*M.c.*) contains two genes for two methane monooxygenases, a particulate (pMMO) and a soluble (sMMO) enzyme. Only one of the two MMOs is expressed at a time, and the single factor regulating enzyme expression was found to be the copper-to-biomass ratio. When the level of copper ions is high, the pMMO is expressed, whereas at very low levels of free copper ions, the bacteria switch to the synthesis of the sMMO. Transcription of the MMO genes is known to be regulated by copper. The soluble methane monooxygenase exhibits a striking lack of substrate specificity, resulting in the fortuitous metabolism of a very large number of compounds including xenobiotic chemicals, especially chlorinated aromatic hydrocarbons. Because of the ability of sMMO to catalyse a large number of biotransformations, it has attracted the interest of scientists involved in the development of biological methods for degradation of toxic chemicals and in the use of bacteria containing MMOs for the production of chemicals with commercial value, for example, primary alcohols and epoxides. Considering the growing number of microbial genome sequencing projects, in-depth molecular biological examination of a diverse range of bacteria is expected in the future.

To analyse the functions of the genes of the increasing number of prokaryotes with sequenced genomes we need to adapt the classical genetic and molecular techniques of the model organisms such as *E. coli* to these, often "difficult" bacteria. Investigation of methanotrophs in the past few decades indicated the high biotechnological potential of these organisms. *M.c.* is a well known, moderately thermophylic methanotroph and its genome is being sequenced at TIGR.

My work concentrated on the optimisation of a conjugation system for *M.c.* which is then used to introduce different vectors into the cells such as broad host range cloning vehicles, transposon delivery and suicide vectors. Practical use of the developed techniques was demonstrated in the investigation of copper regulated expression of the MMOs. Downstream from the sMMO structural genes we have found putative regulatory genes, which highly homologous to the so-called two-component regulatory system genes (*mmoQ-mmoS*), a s^{54} dependent transcription factor homologue (*mmoR*) and a chaperonon homologue (*mmoH*). Mutants were created by marker exchange mutagenesis for these genes. The mutants were characterised concentrated on their influence on the expression of the sMMO. We found that each gene required for the production of active sMMO. The promoter of the sMMO gene cluster was mapped with a transcriptional fusion vector containing the green fluorescent protein as reporter gene.

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DISSERTATION SUMMARY

Isolation of hydrogenase deficient mutants in purple sulfur photosynthetic bacteria by transposon mutagenesis

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Hydrogenases catalyze the reversible oxidation of molecular H_2. Two major classes are distinguished: Fe-only hydrogenases and [NiFe] hydrogenases. The latter is typically composed of an electron transfer small subunit and a catalytic large subunit. The formation of an active [NiFe] hydrogenase involves a complex maturation process. The major steps include the biosynthesis of the unprocessed, inactive subunits, incorporation of Fe-S clusters into the small subunit, the assembly of Ni, Fe and the diatomic ligands (CN, CO) into the active center in the large subunit, and the C-terminal cleavage of the large subunit by a specific protease. Without the action of the pleiotropic (Hyp) maturation proteins (HypA, HypB, HypC, HypD, HypE, and HypF) and the specific protease, maturation stops.

A conjugation based gene transfer system, site directed mutagenesis and random transposon mutagenesis system was optimized for the purple sulfur phototrophic bacterium, *Thiocapsa roseopersicina* BBS. This bacterium has at least three hydrogenases. Screening for hydrogenase deficient phenotypes resulted in the isolation of six independent mutants in a miniTn5 library. In the first class of mutants only the Hyn (hydrogenase 1) activity was affected. The second class of mutants had pleiotropic mutations affecting all hydrogenases. One of the pleiotropic mutations was in a gene showing high sequence similarity to HypF proteins in other organisms. This mutant was further characterized in *in vitro* and *in vivo* experiments. The reconstructed *hypF* gene was able to complement the *hypF* deficient mutant of *T. roseopersicina* BBS. Heterologous complementation experiments, using *hypF* strains of *T. roseopersicina*, *E. coli*, and *R. eutropha*, and various *hypF* genes, were performed. Heterologous complementation was successful in all of the cases tested, although for the *E. coli* host the regulatory region of the foreign gene had to be replaced in order to achieve partial complementation. To characterize the products of the genes isolated, attempts to create a homologous expression system with affinity-tags were made. Expression of a reporter protein was demonstrated, and optimization of purification proce/ dures will be also presented.

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DISSERTATION SUMMARY

The role and regulation of *cycHJKL* operon in *Sinorhizobium meliloti*

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Rhizobium-species are soil bacteria, able to form symbiosis with the leguminous plants. On the roots of the host plant, some symbiotic nodules will appear, where *Rhizobium* bacteroids will fix the atmospheric dinitrogen into ammonia. In our laboratory we are doing research work on *Sinorhizobium meliloti*, the symbiotic partner of alfalfa *(Medicago sativa)*.

During my work I was studying the role and regulation of *cycHJKL* operon from *Sinorhizobium meliloti*. The role of the proteins coded by this operon is the covalent binding of haem prostethic group to the c-type cytochrome apoprotein in the bacterial periplasm. After their biogenesis, the functional c-type cytochromes will remain in the periplasmic space, or will be builded in the membranes, having important role in different electron transport chains. The existence of a symbiotic electron transport chain, which is able to function during the microaerobic conditions of the symbiotic nodules, can produce the necessary ATP level for the energetically expensive nitrogen fixation, was published in 1993. The symbiosis-specific *cbb3*-type terminal oxidase complex contains c-type cytochromes and coded by the *fixNOQP* operon. During our experiments we demonstrated that the expression of our *cyc* operon is also induced microaerobically, like expression of *fixNOQP*, so it has an important role in the symbiosis. We showed that in our case sensing of the low oxygen level is not the responsibility of the well-known fixL/J two-component signal transduction system. We did not find any kind of oxygen sensor, which may have a role in the microaerobic induction of *cyc* operon. Searching the expression level of *fixNOQP* operon in *cyc* mutant bacteria, we find that this level can be higher or lower than in the wild type strain depending on the oxygen level present in the culture. By testing with triphenyltetrazolium chloride, we saw that under microaerobiosis the reduction capacity of *cyc* mutants is lower than in the wild type strain, so we hypothesized that the expression of *fixNOQP* operon under microaerobic conditions is regulated not only by FixL/J, but there is another regulatory system which senses the redox state of the cell.

The CycHJKL proteins are located in the membranes between the bacterial cytoplasm and the periplasmic space. Many laboratories are doing research on their exact role in the biogenesis of c-type cytochromes. CycH protein between its two transmembrane domains has a cytoplasmic loop and a long C terminal domain in the periplasmic space. Previous experiments show that CycH has a role in keeping the c-type cytochrome apoprotein until the covalent binding of the haem group established. In other species it was proved that the C terminal periplasmic part of CycH is functionally different from the other pars of the protein. In our experiments we saw that if there is an *in frame* mutation in one of the *cyc* genes, the biosynthesis of haem in the cytoplasm will be disturbed, and protoporphirin IX, the precursor of haem, is accumulated. If we made a mutation in the C terminal periplasmic part of CycH, there will be no changings in the level of PPIX. We have concluded that CycJ, CycK, CycL, proteins and the N terminal region of CycH, until the PP2982 mutation are nescessary for the normal biosynthesis of haem.

We found three domains on the C terminal of CycH: a tetratricopeptide (TPR), a hydrophobic helical stretch, and a methyl-coenzyme M reductase (MCR) domain. The role of these domains is known in other proteins, and with different complementation experiments we are studying their role in the biogenesis of c-type cytochromes.

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DISSERTATION SUMMARY

Examination of biochemical and molecular genetic background of ochratoxin production in *Aspergillus* species

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Ochratoxins (especially ochratoxin A, OA) are economically important mycotoxins produced by *Aspergillus* and *Penicillium* species. The main aim of our project is to examine the spread of OA production among *Aspergillus* species, and to clarify the biochemical and genetic background of ochratoxin biosynthesis in order to develop a molecular detection method for ochratoxin producing fungi. Phylogenetic analysis of OA producing isolates was also carried out using ITS sequences and other features. The examined taxa involve *Aspergillus* sections *Circumdati*, *Flavi* and *Fumigati*. Phenotypic features and sequences of the intergenic transcribed spacer regions and the 5.8 S rRNA gene of type or neotype strains and other isolates of the 17 species currently assigned to *Aspergillus* section *Circumdati* and some potentially related species indicated that *Aspergillus* section *Circumdati* is paraphyletic. *Aspergillus campestris*, *A. lanosus*, and *A. dimorphicus* with *A. sepultus* were found to be more closely related to *Aspergillus* sections *Candidi*, *Flavi* and *Cremei*, respectively. *A. robustus* and *A. ochraceoroseus* were found not to be related to any of the species examined. Species of the proposed revised *Aspergillus* section *Circumdati* formed two main clades, which could also be distinguished based on phenotypic methods. OA producing isolates were scattered on the dendrogram. A similar study of species of *Aspergillus* section *Flavi* indicated that these species form distinct clades. The three main clades identified based on sequence data could also be distinguished based on colony colour, and their ubiquinone systems (*"A. flavus"*, *"A. tamarii"* and *"A. alliaceus"* clades). The synnematous species *A. coremiiformis* was closely related to species in the *"A. tamarii"* clade. Three species, *A. nomius*, *A. avenaceus* and *A. leporis* were found to form separate lineages not closely related to any of the main clades identified. The intraspecific variability of the *Aspergillus viridinutans* species and its relatives was examined using various techniques including morphological examinations, carbon source utilization tests, restriction enzyme analysis of the mitochondrial and nuclear DNA, and sequence analysis of part of the β-tubulin gene. The ochratoxin A producing *A. viridinutans* strain IMI 306135 was most closely related to an asexual isolate. These two latter strains were more closely related to *A. fumigatus* and *N. fischeri* than to any *A. viridinutans* strains, and possibly represent a new species in *Aspergillus* section *Fumigati*.

Examination of the mycoflora of agricultural products led to the conclusion that *A. ochraceus* is not the only source of OA contamination in these products. Kinetics of ochratoxin A production was also examined in a number of ochratoxin producing isolates representing different sections of the *Aspergillus* genus. Both weak and high ochratoxin producers were tested using immunochemical or HPLC methods. All isolates were found to produce the highest amounts of ochratoxin A after 7-10 days of incubation. The *A. albertensis* and *A. melleus* isolates examined were found to produce ochratoxin A constitutively. Ergosterol content and ochratoxin production of *A. albertensis* cultures were in good correlation.

OA degrading activities of large numbers of Aspergilli were also tested. An *A. niger* isolate was selected for further studies, which could degrade OA and ochratoxin A. Further studies are in progress to apply this isolate or its enzymes for OA decontamination.

OA non-producing mutants of *A. albertensis* were also isolated and characterized. In the future, OA producing and non-producing variants of *A. albertensis* are planned to be screened by RAPD using large numbers of random primers, with degenerate primer pairs based on sequences of polyketide synthase domains, and by the differential display technique. The differentiating DNA fragment would be cloned, characterized and used as a probe against a gene bank of an OA producing *A. albertensis* isolate. The hybridizing clones would be further characterized. Finally, a DNA probe would be developed for fast molecular detection of OA producing fungi in foods and feeds.

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DISSERTATION SUMMARY

Analysis of a novel CDPK kinase in *Arabidopsis thaliana*

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The *Arabidopsis PRL1* (pleiotrop regulatory locus) gene encodes a regulatory WD protein that plays an important role in carbon metabolism, cytokinin and glucose signaling, cell cycle, photosynthesis, and general stress responses (Németh et al. 1998). The CRK1 protein kinase was isolated as a PRL1 interacting partner in yeast two hybrid system. The CRK1 protein is an atypical member of the Ca^{2+}-dependent protein kinases (CDPKs). CDPKs form a large subfamily of protein kinases in plants that have been implicated in the control of numerous aspects of plant growth and development. The CDPKs have four well characterized conserved motifs: 1) ATP-binding; 2) catalytic domain; 3) an autoinhibitor is predicted in the region immediately following the kinase domain; 4) number of functional calcium-binding EF-hands in a C-terminal regulatory domain. In the CRK subgroup, these EF-hands are degenerated. Until now 28 different CDPKs and 8 CRKs have been identified in *Arabidopsis*.

The function of the CRK1 kinase is not known. We use different molecular and genetical approaches to characterize the CRK1 function in plants. Screening of different cDNA libraries in yeast two hybrid system (whole plants, and cell suspension library) resulted in the identification of further interacting partners of the CRK1 protein. From cell suspension library: dihydroflavonol reductase with epimerase domain, two others CDPK-related protein kinase, ER-type calcium ATP-ase, unknown protein (SEC14 domain); from whole plants library: centromer protein homologue, photosystem I subunit II precursor, unknown protein (bZIP domain).

Biochemical characterization of the CRK1 protein has been initiated. A loss-of function mutation was generated by PCR mutagenesis by eliminating an ATP-binding site in the conserved kinase domain (CRK1m). In order to analyze the kinase activity of the CRK1 protein, His-tagged recombinant CRK1 and CRK1m clones were overexpressed in *E.coli*, and the proteins were purified from bacterial extracts. Activity of the wild type and mutant CRK1 protein was tested by phosphorylation of the myelin basic protein (MBP) as a hypotetical substrate. CRK1 but not the CRK1m protein was able to phosphorylate MBP in a calcium-independent fashion.

In order to test interaction of the CRK1 and other *Arabidopsis* proteins in plant cells, the CRK1 and CRK1m cDNAs were cloned into HiA epitope tagging vector (Ferrando et al. 2000). These constructs we transformed to GV3101/pMP90RK *Agrobacterium tumefaciens* strain. In a transient expression system, *Arabidopsis* cell suspension was infected by the *Agrobacteria*. Transformed cells were collected five days after the *Agrobacterium* inoculation. Immunolocalization studies suggested the nuclear localization of the CRK1 protein. Salutation of CRK1 kinase-protein complex has been initiated in order to identify the putative interacting members of the protein complex.

Biological function of the CRK1 protein is being studied by creating CRK1 and CRK1m overexpressing transgenic *Arabidopsis* plants and identification of a knock-out insertion mutant using reverse genetic approaches. Full length CRK1 and CRK1m cDNA were cloned into pPCV-type plant expression vectors in sense and antisense orientation. Transgenic plants were generated by an *Agrobacterium*-mediated in-planta transformation method. Insertion mutant was identified by screening of pooled *Arabidopsis* DNA templates, representing 60,000 independent insertions. PCR characterization of the transgenic lines and the tagged CRK1 mutant is in progress.

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DISSERTATION SUMMARY

Isolation of circadian clock mutants in *Arabidopsis thaliana*

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It is a well known phenomenon that some biological processes exhibit periodic changes on a daily time-scale. Some of these maintain periodicity even in the absence of external periodic stimuli which suggests that an internal pacemaker or "clock" exists in the organism. Indeed, genetic mutations were identified which cause defects in the mechanism producing circadian rhytmicity. In several model organisms the molecular basis for circadian clockwork is described in detail. Surprisingly, circadian clock seems to be a good example of convergent evolution: there are no conserved clock elements among organisms in different taxa. However, it is generally accepted that in all organisms special clock proteins generate circadian rhythms through a transcription/translation feedback loop, parts of which are unrelated, but the functions and the architecture of the system is similar. The rhythm generated by the circadian clock is received by several output elements like photosynthetic activity or gene expression. To keep in phase with the external 24 h cycle of the day, the clock can be reset by several environmental factors which percepted and transmitted to the clock by input receptors.

Despite of the large amount of information available on the molecular nature of circadian pacemaker in other organisms, little is known about the clock mechanism in plants. Therefore we decided to identify the elements of the clock system in *Arabidopsis thaliana* by searching for mutants displaying altered circadian phenotype. We used transgenic lines carrying a Cab2:luc reporter gene for mutagenesis by EMS and T-DNA. We screened for mutants by continuously measuring the luciferase enzyme activity in individual plants over two days in constant darkness after a 7-day long entrainment by 12 h dark-12 h light period. Progeny of individual mutants was tested for herited mutant phenotype either in constant darkness and red light. Mutants exhibited any kind of circadian phenotype were kept for further analysis and mapping. We started the genetic mapping of the mutants showing the most robust phenotype.

After screening of 60,000 EMS mutagenized seedlings we obtained 37 potential mutants. Eight of these were selected for genetic mapping. From the T-DNA screen 5 of the 5,000 line tested were selected as a putative mutant. In none of the 5 cases could we show cosegregation of the T-DNA tag with the mutant phenotype, therefore these mutants will be mapped. After obtaining a rough map position from some EMS mutants we found that it is the same in 3 of our mutants and it is the position where an already described clock gene – ZTL – is located. Therefore, we decided to sequence that gene in the 3 mutants. All of them proved to be new alleles of the ZTL clock gene. ZTL is an F-box protein, which probably plays a role in the ubiquitin-mediated destruction of clock proteins. It contains a LOV/PAS domain, which is tought to bind flavin cofactors or mediate protein-protein interactions and can be found in several clock-associated protein. It contains the F-box which docks target proteins to the E3 ubiquitin ligase complex, and a Kelch-repeat domain which is a common protein-interacting domain and expected to bind the target protein. In one of our mutants, there is an early STOP codon in the last Kelch-repeat, and probably it causes a loss-of-function phenotype. The second ZTL mutant contains a mutation in the 4. Kelch-repeat which causes a non-conservative Gly->Asp amino-acid change, and it is likely a loss-of-function allele, also. The third ZTL mutant mutation is located in the LOV/PAS-domain causing a Leu->Ser amino-acid change. It has an interesting phenotype, because like other ZTL mutants it has a long-period but the difference in period length is constant over a large scale of light intensity which is not true in the case of null-alleles of ZTL. This indicates that in this mutant light-sensitivity remains unaffected, but the function of the molecule is damaged in some extent. Several mutants have not been mapped yet, but have interesting phenotypes and therefore we can expect them to have mutations in unknown clock genes.

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DISSERTATION SUMMARY

Study of two overlapping genes in *Drosophila melanogaster*

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From an independent screen we isolated two *Drosophila melanogaster* genes. Based on homology searches we named one of the genes *dada2a/drpb4*. The other gene got the *dtat* name from its ability to bind the HIV TAR RNA. The *dada2a/drpb4* gene codes for two different proteins through alternative splicing. The dRPB4 protein is the 4th largest subunit of the RNA polymerase II, which attaches to the holoenzyme only in stress conditions in budding yeast. In the fission yeast the protein is a stable member of the RNA polymerase II, and probably this is the docking site for the C-terminal Domain phosphatase of the RPB1 protein. The *dada2a/drpb4* gene codes for another protein, the dADA2a. This is an adaptor protein, which has its yeast homology too. In the yeast this protein is a member of two adaptor complexes, the ADA and the SAGA complexes. They act like a bridge between the transcription factor and the RNA polymerase II, mediating the effect of the transcription factor. In the fruitfly, there are two ADA2 proteins, the dADA2a and the dADA2b. The first was isolated in our laboratory, the second in a cooperating French laboratory. The dtat gene codes for a protein of unknown function, although there are similar proteins in many species found in the database. My goal was to investigate the arrangement of the two genes in the Drosophila melanogaster genome, to examine their transcription regulation and to find out something from the function of the dTAT protein.

As I mentioned the *dada2a/drpb4* gene codes for two types of mRNAs, the dada2a and the drpb4 mRNA. The first exons of these mRNAs are common and the further exons of the dada2a mRNA are located in the first intron of the drpb4 mRNA. The dada2a mRNA itself has two splice variants, they differ in the length of the 2nd exon, but this difference is not shifting the open reading frame. The importance of these two dADA2a variants is not known yet.

The two genes are transcribed in opposite orientation, and they are in head-to-head arrangement. Further studies, including primer extensions, 5'RACE and RNase protection assays, revealed that the genes are overlapping with each other.

The tight arrangement of these genes might suggest a common transcriptional regulation. The observed overlapping transcription prompted us to investigate the possible involvement of a novel gene-silencing phenomenon, the RNA interference (RNAi) in the transcriptional regulation of these genes. RNAi was described in many species including *Drosophila*, and if it is triggered by the introduction of a synthetic dsRNA, it can evoke the degradation of the corresponding mRNA.

To test RNAi, ^{32}P-labelled ssRNAs from different regions of the overlapping genes were synthesised and treated with *Drosophila* embryonic extract. The specific degradation of the labelled ssRNA might indicate the induction of RNAi due to the in vivo appearance of the dsRNA. We observed degradation of ssRNAs corresponding to the overlapping parts of these genes. Control RNA from a closely spaced adjacent gene without overlapping transcription was not degraded.

Our results suggest that RNAi can also be triggered by endogenous dsRNA, and it can regulate endogenous genes by post-transcriptional gene silencing.

To find the function of the dTAT protein we identified its domain structure. It showed that it has an S-adenosine methionine binding domain, but we could not prove that it binds to S-adenosine methionine. From the screen in which we identified the protein we knew that it could bind RNA. We also verified this observation with RNA binding assay. We made yeast-two-hybrid screen to identify the interacting partners. We found interaction between the mouse homologue protein and the human Embryonic Ectoderm Development protein. This protein is a polycomb-like protein, which plays a key role in the embryonic development. It has a homologue in the *Drosophila melanogaster*, the extra sex combs (esc), and genetic studies in the fruitfly underlined the interaction between the dTAT and the esc.

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DISSERTATION SUMMARY

The chondrogenic master transcription factor Sox9 binds to the regulatory region of matrilin-1 gene

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Sox9 is a high-mobility-group (HMG) DNA-binding domain transcription factor which plays an essential role in chondrogenesis and in transcriptional regulation of the type II collagen gene. In humans, *SOX9* haploinsufficiency results in campomelic dysplasia, a lethal skeletal malformation syndrome with XY sex reversal. Furthermore, in chimera studies *Sox9*$^{-/-}$ cells were unable to express chondrocyte-specific extracellular matrix genes. Matrilin-1 (also known as CMP-cartilage matrix protein), one of the major non-collagenous proteins in most cartilages, binds to aggrecan and type II collagen. It is a homotrimer of 54-kDa subunits assembled via a coiled-coil a-helix and stabilized by disulfide bridges. Matrilin-1 is expressed almost exclusively in chondrocytes, and may function as a bridging molecule between two major macromolecular networks of cartilage.

The understanding of the transcriptional regulation of the gene is still limited. To get further insight into the transcriptional regulation of the gene and to analyze protein-DNA interactions *in vivo*, we performed *in vivo* footprinting on the chicken matrilin-1 gene. Ligation-Mediated PCR technique (LMPCR) was introduced that allows the amplification of DNA fragments between positions –227 and +140 and detection of control elements. Chicken embryo chondrocytes (CEC) in comparison with chicken embryo fibroblasts (CEF), the non-expressing cell type, were subjected to *in vivo* analysis in which the total genomic DNA underwent a specific cleavage procedure carried out inside the cell. Sets of experiments revealed tissue-specific binding of transcription factors to the promoter and intronic control regions. For example, a cartilage-specific protection was observed at an inverted repeat carrying two putative Sox9-binding sites in the promoter upstream region of the matrilin-1 gene. To provide evidence that Sox9 is indeed involved in the transcriptional regulation of the matrilin-1 gene, we designed synthetic oligonucleotides (wild type and several mutant ones) for gel retardation assays. *In vitro* analysis using electrophoretic mobility shift and supershift assays confirmed the binding of Sox9 transcription factor to the identified control element. Furthermore, using *in vivo* footprinting, we could prove tissue-specific binding of NFI family transcription factors to the silencer elements SI and SII identified previously.

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DISSERTATION SUMMARY

Study of structure-function relationship of human galectin-1

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Lectins are proteins which specifically bind (or crosslink) carbohydrates. Galectins are defined as lectins having both galactose-binding ability and characteristic conserved amino-acid sequence. Galectin-1 is a homodimeric lectin with specificity for beta-galactosides. It is found in many species from fungi to mammals. In mammals its expression is developmentally regulated in a variety of organs from brain to thymus. Galectin-1 has been shown two participate in cell adhesion and growth, immunomodulation, apoptosis, metastasis, inflammation, and premRNA splicing. It has been proposed to play important roles by recognizing carbohydrate moieties on intracellular ligands, cell signaling receptors and extracellular glycoproteins, although precise knowledge of the mechanism of galectin-1 action is lacking. To reveal this mechanism we are conducting a structure-function study of the lectin.

We applied two approaches in our research. The first one is to study the extracellular function of lectin. cDNAs of wild type and carbohydrate non-binding mutant of galectin-1 were cloned into pET-His bacterial expression vector. Obtained recombinant proteins were successfully purified by affinity chromatography on Ni-NTA agarose utilizing 6xHis tag. Wilde type and mutant his-tagged proteins showed the similar properties in polyacrylamide gel electrophoresis and western blot analysis to the normal non-his tagged recombinant galectin-1. These proteins were tested for ability to induce apoptosis in human lymphoid T-cell line Jurkat. Galectin-1 and his-galectin-1 induced a similar degree of apoptosis in tested cell line. His-tagged carbohydrate non-binding mutant of galectin-1 did not induce apoptosis. This result can lead to a conclusion that apoptosis-inducing function of galectin-1 is dependent on its beta-galactoside binding ability. Other mutant forms of galectin-1 will be produced as recombinant proteins and similarly tested.

Second approach is to study intracellular function of the lectin. With this goal cDNA of wild type and mutant galectin-1 were cloned in mammalian retroviral expression vector pLXSN. These constructs in parallel with empty vector for control were introduced into Jurkat, BL-41, and Daudi cell lines by retroviral transfection. Successful transfection was confirmed by western blotting. Jurkat cell lines were analyzed further. Cell surface markers analysis by fluocytometry revealed no differences between cell lines expressing and non-expressing galectin-1. Existing data on participation of galectin-1 in processes of cell growth prompted us to conduct analysis of proliferation of transfected cells. In MTT proliferation assay cell line expressing wild type galectin-1 showed significantly lower proliferation rate than cell lines expressing mutant or no galectin-1. Again, as in case of apoptotic function of recombinant protein, this function of galectin-1 seems to be dependent on its beta-galactoside binding activity. Further experiments are needed to confirm this result. Participation of intracellular galectin-1 in regulation of apoptosis is of particular interest. Future experiments will test sensitivity of transfected cell lines to apoptosis by different agents including galectin-1 itself, anti-CD95 antibody, staurosporine and others. Galectin-1 has been shown to participate in T-cell receptor (TCR) signal transduction. Response of transfected cells to the activation through TCR will be studied in different aspects (calcium mobilization, tyrosine phosphorylation, phosphatase activity).

Althoug more experiments have to be done, already completed studies of recombinant proteins and transfected cell lines prove the dependence of galactin-1 function on its beta-galactoside binding ability. Its intracellular presence in many cell types suggests the importance of cytoplasmatic and nuclear protein glycosilation. Recent studies show that, indeed, some of the intracellular proteins are glycosilated *in vivo*.

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DISSERTATION SUMMARY

Embryonic development of the human enteric nervous system and the enteric microenvironment

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The enteric nervous system (ENS) is large, complex and independent of the central nervous system. Its neural-crest-derived precursors migrate along defined pathways to colonize the bowel. It has been established that signalling molecules produced by the developing neurons and the mesenchyma of the gut wall play a critical role in the development of the mammalian ENS (Furness and Costa 1987).

Since the morphological and neurochemical properties of the enteric plexuses are various in different species, the investigation of human fetal material is necessary in order to establish the basic rules of the development of the human ENS. All experiments with human fetal material were performed in accordance with the declaration of the Medical World Federation proclamed in Helsinki in 1964.

Morphometric analysis of the tissue layers of human gut wall

The ENS develops in close correspondence with the enteric microenvironment (Fekete et al. 2000). Throughout present investigation, the qualitative and quantitative changes in the tissue layers around the intestinal lumen were investigated in the different segments of the developing human fetal gut at weeks 12 and 18 of gestation using light and electron microscopic techniques. Two main questions were raised during studying the fetal development: is there any correlation between the development of the tissue layers forming the gut wall? Is there any regional difference between the development of tissue layers in the gut wall under the observed period?

Thin and ultrathin sections were prepared from different parts of the human fetal gut. The thin sections were analysed with Image-Pro Plus 3.0 software. Analysis of the data was made by two-way ANOVA and Pearson's-correlation.

The development of the different layers showed various tendencies. The thickness of the epithelia did not change significantly, but the ultrastructural features exhibited remarkable changes. Pearson's correlation revealed correlative development between the change in the thickness of the circular muscle layer and the thickness of the myenteric plexus, but not between the other tissue layers (Bagyánszki et al. 2002a).

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Neurotransmitters and receptors of myenteric neurons in the developing human fetal ENS

The first aim of our work was to determine the individual distribution and colocalization of VIP, NPY, NOS, GABA and glutamate in the developing human small intestine. Since the presence of the glutamatergic neurons in the human ENS was not investigated before, our second aim was to find neurons expressing NMDA receptors and serving as a target of glutamatergic excitatory input.

Wholemounts were prepared from the human fetal gut. Single- and double-labelling immunofluorescence histochemistry were used with antibodies raised against NOS, VIP, NPY, GABA, glutamate and NMDA receptors. The species-specific secondary antibodies used for visualizing immunopositivity were conjugated to Cy3, TRITC or FITC.

NOS-immunopositive neurons were numerous whereas VIP-, NPY, GABA- and glutamate-immunoreactive cells were rarely seen. Double-labelling experiments revealed NOS, GABA and NOS, NPY coexistence, in addition, these experiments demonstrated VIP-immunopositive pericellular baskets around a given population of NOS- and NMDA-immunopositive myenteric neurons (Román et al. 2002; Bagyánszki et al. 2002b).

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DISSERTATION SUMMARY

Plasticity of the somatosensory cortex in adult rodents

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Life is a long history of sensory experience, in the course of which the nervous system proves capable of adapting to the ever-changing environment as well as to behavioural challenges and pathological alterations. This ability is termed plasticity. A fundamental issue in neurobiology is the understanding of the morphological and physiological correlates of these adaptive changes in the nervous system. Controlled alterations of sensory input permit studies of the consequent structural and functional changes, *i.e.* studies of experience-dependent neural plasticity.

In our experiments we have been studying alterations of functional parameters with electrophysiological methods on the so-called barrel field of rodents. This region of the cerebral cortex contains discrete groups of neurons in layer IV, called barrels, which are related one-to-one to the large mystacial vibrissae on the contralateral face. Anteromedially we can find the forepaw representation area adjacent to the barrel field, which is also topographically organized. Despite the strict cortical representation of the body surface on the primary somatosensory cortex (SI), a significant capacity to undergo functional changes in response to alterations in sensory input remains even in the adult cortex.

First, we investigated the consequences of infraorbital nerve injury on the organization of cortical representational maps in adult rats. The infraorbital nerve is a sensory branch of the trigeminal nerve that innervates the whisker follicles of the face, so its injury eliminates the input of the contralateral barrel cortex. Recording somatosensory evoked potentials and extracellular unit activity over the barrel field as well as on the adjacent forepaw representation area we present evidence indicating that changes in the somatotopic map of the SI appear early after nerve crush. We closely studied the borderline between the physiological representation of the sinus whiskers and the digits. Following the injury, the physiological representation of the digits of the contralateral forepaw extended posterolaterally, occupying a part of the whisker region. The extended physiological representation of the digits, though somewhat shrunken, remained after the reappearance of whisker-evoked responses, forming an overlapping area between the obligate digit and whisker representations. Thus, we demonstrate that entire reorganization in cortical topography does not take place after nerve regeneration in adult animals.

Our purpose was then to identify physiological correlates of cortical plasticity, however subtle, that arise as a result of milder, nearly natural alterations of sensory experience, leaving the nervous system intact. So we examined the changes of somatosensory evoked potentials after increased and a subsequent decreased use of the vibrissae in adult mice. Thus, the animals were first subjected to a behavioural challenge, the radial arm maze, which requires active use of the vibrissal system and also motor skills. Their whiskers were then trimmed to create a state of sensory deprivation. Furthermore, to follow activity-dependent changes, their time course or reversibility, it was desirable to apply a technique that allows repeated measurements on the same group of animals. Therefore, we applied the minimally invasive epicranial evoked potential recording method, which proved to be sufficient for repeated use. The consequences of each alteration were measured above the primary somatosensory and motor cortices of the contralateral hemisphere. The latencies of the evoked potentials were found to shorten, while their amplitudes decreased, after the behavioural challenge involving the vibrissal apparatus. Sensory deprivation achieved by whisker trimming resulted in a partial reversal of the changes observed after increased activity. Some derived parameters imply that cortical information processing speeds up as a result of experience, while decreased activity has the opposite effect.

Our results demonstrate that the nervous system is capable of adaptive changes, such as reorganization of cortical maps or change of the dynamics of the evoked potentials at adult age, and these functional alterations are detectable and traceable even by minimally invasive physiological methods through repeated measurements on the same group of animals.

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Symposium

Dedicated to Professor Gyula L. Farkas on the occasion of his 70th birthday

Organized by

Archeology and Anthropology Committee, Szeged Chapter,
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Odontology Committee, Szeged Chapter, Hungarian Academy of Sciences

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Invited Lectures

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SYMPOSIUM

Subjective words to the jubilee of Gyula Farkas*

Ottó Trogmayer

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I have no reason to deny that I am biased as I am trying to organise my thoughts; the thoughts which come to the surface when looking through the work and life of half a century. I am biased and subjective, as the memory of the years of the second half of the century comes into my mind. I remember the atmosphere of the excavations, the hours, when we were sitting together by the telephone, excited about the birth of his daughter, Ildikó. I remember the shared happiness of our successes and the shared bitterness of our failures. I think I have no reason to deny, our work started at the end of the fifties, from which a friendship grew out. I am proud of the work, with which we aimed at reforming the activity of the scholars of archaeology and anthropology on the modern bases of the time. I remember it also how we thought about our teachers who were in their fifties, when we were twenty.

We were both dreaming about an institute where the studies which support each other as auxiliary sciences, are able to answer together the questions of the history of our country, our native land, and the people living here, with the help of consultations and of putting up and immediately answering questions. From these ambitions, the co-operative work grew out. It is true, that you have made long digressions in the fields of anthropology, which deals with the contemporary populations. In addition, here you managed to reach great success, but for me the researches carried out in the field of historical anthropology were the most important. Well, as it turns out from the great many articles translated to different languages, you have made something long lasting. In my opinion your permanent and brave opposing to the fashionable charlatans of the field, who discredit the prestige of our "scientia amabilis" in and out of our country, is a special achievement.

Praising cannot appear without biographical data but I could not find your name in the Hungarian "Who is who". This is not a shame, but the publisher's thoughtlessness, in that it had not listed each professor of each university, who is "a somebody" in Hungary after all. The professors, who receive their commissions not from an association or a party, but from the president of the republic himself.

Gyula (László) Farkas was born in Szabadszállás in Bács-Kiskun County, on April 11, 1932. He completed secondary school in Kecskemét, and the university in Szeged. He graduated at the Attila József University as a teacher of biology and chemistry, in 1954. After having taught half a year in Kecskemét, he came back to the alma mater of the higher education, where he received all the promotions of seniority as teacher and as researcher, too. First an assistant lecturer, than a candidate of biological science in 1976. In 1977, he became a university lecturer, and since 1980, he is the head of the Department of Anthropology in the University of Szeged. In 1988, he became a university professor.

He has had a number of academic offices, and received more rewards for his educational work. The most important pieces of his scientific work has been listed in more detailed biographies and in the almanac of the University of Szeged, so now I will not list the works.

In my opinion, it is true that a scholarly researcher and professor may be qualified after his articles, his life work and his students. This amount is in itself exemplary. We know that most of it has not been written with co-authors, which is not usual in the fields of natural sciences.

The professor has spent most of his life within the walls of the institute. He would not be ashamed of doing physical

*Dedicated to Professor Gyula Farkas on the occasion of his 70th birthday.

work if it was necessary. He has done everything for raising the reputation of his Department, and for keeping the level of education on a high level, for securing the conditions of this. He has kept on trying to introduce new research methods. As a teacher, he has held – according to modest calculations – more than five thousand hours of lectures, on a high level.

In my view, his working capacity and diligence are a great example to be imitated, but can never be caught up with.

He taught generations of biology teachers, who are proud of their master. We can only feel sorry about the fact, that out of his students only a few could manage into such a collection, where they would be able to enter their abilities into the service of research only. This is due to the status of the Hungarian museums.

My dear Gyula! A well-known art historian lady, answering the acknowledgements she received on a similar occasion, asked the people present: "Are you praising me now, or burying me?"

Well, I have ordered my personal thoughts for your seventieth birthday, as a friend, a colleague, to wish you happiness in life, and new results in science. I know that with these wishes, I am not alone. Each member of the Hungarian anthropology and palaeontology wishes the same to you.

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SYMPOSIUM

New findings – new problems in classification of hominids*

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ABSTRACT The criteria for the inclusion of species within the genus *Homo* have changed over the years. There has been a stepwise relaxation of these criteria, therefore the classification and the evolutionary place of hominid fossils have never been free of controversy. It is the main reason that the discoveries of new hominid fossils have not helped in solving the generally accepted classification of hominids. **Acta Biol Szeged 46(1-2):57-60 (2002)**

KEY WORDS

gradistic classification
cladistic classification
phylogenetic classification
primates
hominids

"...neither the paleontological, nor the genetical, nor the archeological records as they now stand can tell us exactly when, where, or how..." (Howells 1967)

Although almost 35 years passed after Howells (1967) wrote the above cited statement concerning the evolution of the hominids, it remained as true as it was earlier.

The species of the hominids that have been recognised since the late Pliocene fossils in the continents of the Old World have always been in the center of a never ending debate: when, where and how they evolved into our species, the *Homo sapiens*.

One of the main reasons of this debate is the lack of consensus concerning the number of the taxa of the hominids and their hypodigms. However, the root of this controversy can be found in the different taxonomies of the order Primates.

The terms "hominoid", "hominid", and "hominin" are not interchangeable, but their classification criteria are variously in a state of flux. In general, the hominoids are a superfamily of Primates; the family *Hominidae* is currently considered to comprise both the great ape lineages and human lineages within the hominoid superfamily; the subfamily *Homininae* comprise both the human lineages and the African ape lineages within the hominids, and the tribe *Hominini* comprising only the human lineages. This current scheme is given in Table 1.

Classification: "...the ordering of (organisms) into groups (or sets) on the basis of their relationships..." (Simpson 1961).

Major changes in the classification of hominids

When the genus *Homo* was introduced in 1758 by Linné, it embraced two extant species. The first one, *Homo troglodytes*, also known as *Homo sylvestris*, is now known to have been based partly on the orangutan, and partly on myth. The second one was the *Homo sapiens*, the species to which all modern human populations belong. Since its introduction almost 250 years ago, our understanding of *Homo* has been changed by the addition of fossil species. This has resulted in the step-by-step relaxation of the criteria for the inclusion of species into the genus *Homo*.

Until the middle of the 1960s, all the classification of the primates were based on Simpson's classification (1945, 1961), which used only morphological characteristics, and a genus may be monopyletic or paraphyletic, too. These kind of classifications divided the superfamily *Hominoidea* into two families: *Pongidae* (for *Gorilla*, *Pan*, *Pongo* and *Hylobates*) and *Hominidae* for *Homo* alone. Among fossil taxa, *Australopithecus* was sometimes allocated to the *Pongidae* (Simpson 1945), sometimes to the *Hominidae* (Le Gros Clark 1959). In the 1960's an increasing trend appeared towards awarding the gibbons their own family, *Hylobatidae* (Napier and Napier 1967).

In 1963, Goodman's immunological study of serum proteins divided the superfamily *Hominoidea* into three branches: the gibbons, the orangutan, and an irreducible cluster of human, gorilla and chimpanzee. This can be recognised in taxonomy, with the families *Hylobatidae*, *Pongidae* (restricted to the orangutan) and *Hominidae* (for *Homo*, *Pan* and *Gorilla*). Molecular evolutionary techniques have progressed from immunology through aminoacid sequencing, DNA-DNA hybridisation, RFLP to DNA sequencing, but all have confirmed the same basic groupings, merely refining the trifurcations, so it is now evident that the gibbon line did diverge before that of orangutan, and most studies have concluded that the gorilla diverged before the human and chimpanzee lines separated.

However, the contradiction between these two classifications is only apparent. Groves (1986) collected numerous morphological characteristics and found that, when analysed cladistically, the morphological analysis produced exactly the same phylogeny as the molecular ones (Table 2).

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Table 1. Current scheme of the primate superfamily.

Superfamilia *Hominoidea*
- Familia *Hylobatidae*
- Familia *Pongidae*
- Familia *Hominidae*
  - Subfamilia *Homininae*
    - Tribe *Gorillini*
    - Tribe *Hominini*
      - Genus *Ardipithecus*
      - Genus *Australopithecus*
      - Genus *Paranthropus*
      - Genus *Kenyanthropus*
      - Genus *Homo*

The other classification is the cladistic one and in this interpretation of classification a genus must be monophyletic; it cannot be paraphyletic.

"...evidently, evolution at the structural gene level and at the morphological level do not obey the same rule." (Ney and Roychondhury 1982).

The cladistic taxonomy

Although the above cited statement is true, when the morphological characters and the molecular ones are analysed cladistically, they produce the same phylogeny (Groves 1986), as can be seen in Table 2.

The founder of cladistic taxonomy was Hennig (1966), who observed that one of the more perplexing problems in taxonomy is the assigning of ranks to the groups in a hierarchical taxonomic classification. A partial solution to this problem is to have phylogenetic classifications in which all taxa represent monophyletic groupings, *i.e.* the names of the taxa can serve as the names of actual clades. Clearly, on so naming clades, a younger clade nested within an older clade, taxonomically must always have a rank at a lower hierarchical level than the older clade. For example, all taxa with the rank of family in a cladistic phylogenetic classification of primates should be of younger age than the order Primates, the older more inclusive taxon; similarly all orders of mammals should be of younger age than the class *Mammalia*. In as much as ranking solely by relative age does not ensure that taxa assigned the same rank represent clades that are equivalent to one another with respect, at least, to some key objective measure. Hennig (1966) reasoned that the optimal yardstick for measuring which clades are equivalent is the absolute age of origin of the clades, *i.e.* the taxa assigned the same rank should represent clades of about the same absolute age. Perhaps because such a temporal system of classification would be inordinately difficult to achieve across phyla, Hennig (1981) initiated a trend among cladists to abandon the use of ranks altogether. Nevertheless since long established rules in the practice of taxonomy require that taxonomic names with the endings *oidea*, *idae*, *inae*, *ini*, and *ina* designate the ranks of superfamily, family, subfamily, tribe, and subtribe, respectively, and since most systematists and

Table 2. Phylogenetic classification of primates (modified after Goodman et al. 2001).

Semiorder *Strepsirhini* (50 My)
- Suborder *Lemuriformes* (45 My)
- Suborder *Loriformes* (23 My)

Semiorder *Haplorhini* (58 My)
- Suborder *Tarsiiformes* (?)
- Suborder *Anthropoidea* (40 My)
  - Infraorder *Platyrrhini* (26 My)
  - Infraorder *Catarrhini*
    - Superfamily *Cercopithecoidea* (25 My)
      - Family *Cercopithecidae*
      - Family *Hominidae*
      - Subfamily *Homininae* (18 My)
        - Tribe *Hylobatini*
        - Tribe *Hominini* (14 My)
          - Subtribe *Pongina*
            - *Pongo pygmaeus*
          - Subtribe *Hominina* (7 My)
            - *Gorilla gorilla*
            - *Homo* (6 My)
            - *H. (Pan)* (3 My)
            - *H. (Pan) troglodytes*
            - *H. (Pan) paniscus*
            - *H. (Homo) sapiens*

The age (in million years) is shown in parentheses.

taxonomists still use ranks in their classifications, Hennig's cogent reasons for a rank equals age system of phylogenetic classification still have merit. Moreover, molecular phylogenetic investigations have provided tools along with those of paleontological investigations for dating branch-points in phylogeny and thus for constructing phylogenetic classifications in which taxa at the same rank represent clades of equivalent age (Goodman et al. 2001).

Table 3. A genealogical classification of extant and extinct species within the genus *Homo* (Goodman et al. 2001).

Homo
- *H. (Pan)*
  - *H. (P.) paniscus*
  - *H. (P.) troglodytes*
- *H. (Homo)*
  - *H. (H.) ramidus*
  - (*Ardipithecus ramidus*, 4.4 My)[a]
    - *H. (H.) anamensis*
    - (*Australopithecus anamensis*, 4.2-3.9 My)[a]
  - *H. (H.) afarensis*
  - (*Australopithecus afarensis*, 3.6-2.8 My)[a]
    - *H. (H.) africanus*
    - (*Australopithecus africanus*, 2.8-2.4 My)[a]
      - *H. (H.) boisei*
      - (*Australopithecus boisei*, 2.4-1.3 My)[a]
      - *H. (H.) robustus*
      - (*Australopithecus robustus*, 2.0-1.6 My)[a]
    - *H. (H.) habilis*
    - (*Homo habilis*, 1.9-1.8 My)[a]
      - *H. (H.) erectus*
      - (*Homo erectus*, 1.8-0.9 My)[a]
        - *H. (H.) sapiens neanderthalensis*
        - (*Homo neanderthalensis*, 0.5-0.1 My)[a]
        - *H. (H.) sapiens sapiens*
        - (*Homo sapiens*, 0.5-0.0 My)[a]

[a] Shown in parenthesis is the species' name and age from Yoon (1995) for each species that are treated as a member of subgenus *Homo (Homo)*.

Molecular phylogenetic investigations utilize the knowledge that each present-day genome contains a range of DNA sequences from rapidly to extremely slowly evolving. This makes it possible to discover the phylogenetic relationships that exist among living species at all levels of the taxonomic hierarchy from the most recently to the most anciently separated. The advances in doing so are bringing about a two-fold shift in paradigms, one in systematics and the other in how we humans should view our place in nature. The new paradigm in systematics is essentially that first envisioned by Charles Darwin and further developed in a rigorous scientific way by Willig Hennig. It calls for disbanding the use of so-called grade taxa, such as the traditional primate taxa *Prosimii* and *Pongidae* with their paraphyletic groupings and instead calls, as sketched out above, for strictly genealogical (*i.e.* cladistic) classifications that depict sister-group relationships and, ideally, denote by rank level the clades of equivalent age. The other new paradigm rejects the traditional anthropological view that humans are greatly different from all other animal species. Instead, the molecular view emphasizes how much humans hold in common with other species, especially with our sister-group the common and bonobo chimpanzees. Table 3. presents, in terms of the DNA and paleontological evidence on primate phylogeny, the phylogenetic classsification of hominids based on the work of Goodman et al. (2001).

"Human evolution is like a bush, not a ladder" (Gould 1977).

New findings of hominid fossils – new problems of taxonomy of hominids

One of the main reasons of the different interpretations of the evolutionary way of the hominids is that the classification and the evolutionary place of hominid fossils has been under constant debate. It is caused partly because hominid fossils are not plentiful – inspite of the growing number of the fossils – and perhaps partly because there are a number of rival discovery teams, and the importance of a new hominid fossil discovery is enhanced if the discovery apparently requires new classifications and/or new interpretations.

The criteria for the inclusion of species within the genus Homo have changed over the years. The tendency has been for stepwise relaxation of these criteria, moreover, the last revision of the boundaries of the genus *Homo* happened several years ago.

In practice, there are four commonly used criteria for allocating individual fossils to species of *Homo*, and three of

Table 4. List of the current species of hominids.

Species	Type specimen	Named by
Orrorin tugenensis	BAR 1000'00	Senut et al. 2001
Australopithecus ramidus		
Ardipithecus ramidus	ARA-VP 6/1	White et al. 1994
Australopithecus anamensis	KP 29281	M. Leakey et al. 1995
Australopithecus afarensis	KT 12/H1	Johanson et al. 1978
Homo antiquus	AL 288-1	Ferguson 1984
Australopithecus bahrelghazali	KT 12/H1	Brunet et al. 1996
Kenyanthropus platyops	KNM-WT 40000	M. Leakey et al. 2001
Australopithecus africanus	Taung	Dart 1925
Australopithecus garhi	BOU-VP-12/130	Asfaw et al. 1999
Paraustralopithecus aethiopicus		
Australopithecus aethiopicus	Omo18	Arambourg & Coppens 1968
Paranthropus robustus		
Australopithecus robustus	TM 1517	Broom 1938
Australopithecus walkeri	KNM-WT 17000	Ferguson 1989
Zinjanthropus boisei		
Australopithecus boisei	OH 5L.	Leakey 1959
Paranthropus crassidens		
Australopithecus crassidens	SK 6	Broom 1949
Homo antiquus praegens		
Australopithecus praegens	KNM-T1 13150	Ferguson 1989
Homo habilis	OH 7	L. Leakey et al. 1964
Homo louisleakeyi	OH 9	Kretzoi 1984
Pithecanthropus rudolfensis		
Homo rudolfensis	KNM-ER 1470	Alexeev 1986
Homo microcranous	KNM-ER 1813	Ferguson 1995
Homo ergaster	KNM-ER 992	Groves & Mazak 1975
Pithecanthropus erectus		
Homo erectus	Trinil 2	Dubois 1894
Homo antecessor	ATD6-5	Bermudez de Castro et al. 1997
Homo heidelbergensis	Mauer 1	Schoetensack 1908
Homo rhodesiensis	Kabwe	Woodward 1921
Homo helmei	Florisbad	Dreyer 1935
Homo neanderthalensis	Neandertal 1	King 1864
Homo sapiens	–	Linnaeus 1758

them are connected with performance or technical competence. These are 1) the ability to manufacture stone tools, 2) the related possession of modern human-like precision grip (Leakey et al. 1964; Tobias 1991), and 3) the language competence (Tobias 1991). However, there is good evidence that these criteria are either impossible to operate within the constraints of the hominid fossil record, or that the competencies they refer to can no longer be confidently restricted to *Homo* (Gannon et al. 1998). The fourth one, 4) the absolute brain size, is only to be based directly on morphological evidence, but even this has been shown to be of questionable biological significance (Martin 1983).

The chaotic state of the species of genus *Homo* can be recognised in Table 4. Among the several species listed here there are only a few whose taxonomic place or given name are not under debate.

"If taxonomy (above species level) is ever to become more than mere stamp collecting, it must define its spheres of usefulness and examine its philosophical basis. It will be an objective science if it can reflect some part of the real world and if it can be made testable against some other standard..." (Groves 1986).

Conclusions or solutions?

The first step on the long way to reach an agreement should be the determination of the criteria of a genus, then specific criteria for *Homo* have to be generated. Wood and Collard (1999) proposed that a genus should be both a clade and a grade and can be defined as "a species, or monophylum, whose members occupy a single adaptive zone". That means, in case of genus *Homo*, the species within it should be more closely related to the type species, *Homo sapiens*, than they are to australopithecine genera.

Wood and Collard (1999) also suggested based on investigations using both traditional qualitative characters and characters generated from quantitative data that the only fossil species that form a clade with *Homo sapiens* are *Homo neanderthalensis*, *Homo heidelbergensis*, *Homo erectus*, and *Homo ergaster*. This opinion is supported by their body size, body shape, locomotion and diet. The only uncertainty can be seen in the case of *Homo ergaster*, whose relative brain size does not align it so strongly with *Homo sapiens*. On the other hand, according to cladistic and gradistic criteria, *Homo habilis sensu lato*, or *Homo habilis sensu stricto* and *Homo rudolfensis*, are closer to australopithecines than they are to *Homo*. That means that these two species need either to be transferred to an existing australopithecine genus or to be placed to a newly created genus (Wood and Collard 1999).

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SYMPOSIUM

Growth type and motor performance in schoolboys – an international comparison*

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ABSTRACT The aim of the present study was to compare the growth type of 10-13-year-old non-athletic children living in different geographical regions, namely in Cyprus, Egypt, Hungary and Malaysia. Altogether 2,050 volunteer youngsters with less than 25% body fat content were investigated. Body build was estimated by the metric and plastic indices (Conrad 1963), and their cardiorespiratory endurance was assessed by the time of a 1,200 m run. The Hungarian boys were the tallest, and the Egyptians were the smallest, consistently. However, no consistent differences could be observed between the body mass means. The Hungarian boys could be characterised by the most negative metric index means, and the Cypriots were the most picnomorphs. Excluding the significant height differences from the variability of plastic index the consistent differences have disappeared. The most negative metric index means were found at 12 years of age in the Chinese, Cypriot and Egyptian samples, and the average metric indices have become greater consistently at the 13-year-old boys. The mean trend of growth type indices in Hungarian subjects has indicated a significant decrease in accordance with the data published earlier. By the cardiorespiratory endurance the subjects can be divided in two groups. Significantly better performances refer to the Cypriot and Hungarian subjects. The growth type indices introduced by Conrad (1963) sensitively reveal the slight differences in body built and physique resulting from ethnic differences, various physical activity, etc.

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KEY WORDS

metric and plastic indices
1,200 m run
10-13-year-old boys

Anthropometric techniques (for instance, calculation of somatotype components, growth type indices, comparison with the dimensions and parameters of unisex human phantom) for the estimation of physique and body built can be evaluated as relatively new methods.

The Conrad's growth type indices for the description of physique and bone-muscle development (Conrad 1963) are more often used in kinanthropometry. The early publications have come from German (Tittel and Wutscherk 1972) and Hungarian (Szmodis et al. 1976) working groups. The results of Szmodis and co-workers (1976) and Mészáros and associates (1981) proved that successive age group means of metric index (the linearity character of physique) for boys between 7 and 18 years describe a significant second power tendency with peak linearity at 13 years of age both in athletic and non-athletic individuals. In respect of motor functions it was proved repeatedly that the greater bone-muscle development refers to better motor performance and conversely (Mészáros and Szmodis 1977; Szabó and Mészáros 1980; Mohácsi and Mészáros 1986). Unfortunately, no data are available of the growth type characteristics of children and adolescents living in other countries. The only publication in this respect belongs to Othman (2001) who investigated Arabic children.

The aim of the present study was to compare the growth type of 10-13-year-old non-athletic children living in different geographical regions, namely in Cyprus, Egypt, Hungary and Malaysia.

Subjects and Methods

Kinanthropometric data collection was carried out in healthy volunteer boys belonging to the middle socio-economic class in all the four countries. Frequency distribution of the subjects by age and nationality can be seen in Table 1.

The Cypriot boys were the inhabitants of Nicosia and the suburbs of the capital. The Cypriot sample contains only Cypriot-Greek individuals. The Egyptian children were living in Banha city (all of them had Arabic origin) in the northeastern part of the country. This settlement is the capital of Banha County with about 2 million inhabitants. The Hungarian sample contains only a population of children from Budapest and adolescents with Europid origin. Malaysian boys were living in Ipoh (the capital of Perak State) in North Malaysia. The ratio of the three dominant nationalities (Chinese, Indian and Malay) in this settlement is approximately 30-30%. Only children with Chinese origin (both

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Table 1. Frequency distribution of the subjects by age and nationality.

Age	Chinese	Cypriot	Egyptian	Hungarian	Total
9.51-10.50	109	116	128	160	523
10.51-11.50	107	101	114	169	501
11.51-12.50	116	102	115	175	508
12.51-13.50	110	105	113	180	518
Total					
2,050					

parents and grandparents are Chinese) were enrolled into the investigation.

Five physical education (PE) classes were held within 10 days; however, marked differences can be observed between the general content of physical education of the different countries.

Relative body fat content was estimated by the method of Parízková (1961). Since the growth type indices of fat and obese individuals are significantly different than those of the normal ones (Farkas et al. 1999), children with body fat content greater than 25.0% were excluded from this comparison. In this manner, the mean relative body fat content ranges between 16-19% with relative standard deviations of 25%.

Metric and plastic indices were calculated by 6 body dimensions using the equations of Szmodis and associates (1976). The necessary dimensions are: height, shoulder width, chest width and depth, lower arm girth and hand circumference. In taking body dimensions the IBP suggestions (Weiner and Lourie 1969) were observed. All the children were investigated by the working group, girths, width and skinfold thicknesses by the same individual.

Metric index relates the chest width to the chest depth and is corrected by the actually measured stature. At first look, the index is one of the descriptions of the roundness of the chest, however, following its validation the calculated parameter is characteristic for the roundness or linearity of the whole body (Szmodis et al. 1976).

The metric index for boys and males can be calculated as follows:

$$MIX = 0.16\,(CHD - 0.26BH + 0.80CHW - 2.61)$$

$$R = 0.999$$

where: MIX = metric index, CHD = chest depth (cm), BH = height (cm), CHW = chest width (cm), R = multiplied correlation coefficient indicating the congruence between the nomographic and calculated values.

Strongly negative values refer to the leptomorphic (linear) body built, the slightly negative ones to the athletic physique, and the positive ones to the picnomorph constitution.

Plastic index is the arithmetic sum of three body dimensions and it is characteristic for the absolute bone-muscle development.

Plastic index = shoulder width + lower arm girth + hand circumference.

By the numeric values of these two indices a right-angle co-ordinate system can be created, where the vertical axis is scaled by the metric index and the horizontal one refers to the plastic index. The metromorph-normoplastic body built is located at the centre of the co-ordinate system. The upper-left quarter contains the leptomorph but hypoplastic individuals, the upper-right quarter refers to the leptomorph-hyperplastic body built. The lower-left area is characteristic for the picnomorph-hypoplastic constitution, and the lower-right quarter contains the picnomorph-hyperplastic physique variants. The vertical axis is suggested to be positioned to the level of the respective and representative plastic index average in children.

To eliminate the obvious and significant effects of height differences, relative body mass and plastic index were also calculated. Relative measurement = variable x 0.01stature^{-1}.

Cardiorespiratory endurance was estimated by the time of a 1,200 m run. Execution: following the roles of track and field athletics. Reading accuracy was 0.1 s.

Differences between age group means and standard deviations were analysed by F-test following one way ANOVA at 5% level of random error.

Results

Descriptive and comparative statistics for height, body mass and height-related body mass can be seen in Tables 2-4. The Hungarian boys were the tallest (Table 2) in all the four age groups, and the Egyptians were the smallest consistently; however, the differences between the Chinese, Cypriot and Egyptian means were occasionally also significant. The

Table 2. Descriptive and comparative statistics for height (cm).

Group	Chinese		Cypriot		Egyptian		Hungarian		
Age	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P
10	136.52	6.00	137.90	5.86	133.91	7.83	140.31	6.54	<5%
11	141.95	7.23	143.81	6.91	140.77	6.20	146.88	6.74	<5%
12	147.37	8.28	149.04	7.27	146.37	7.14	152.42	6.22	<5%
13	154.06	7.58	157.84	7.67	151.02	7.54	158.02	6.42	<5%

SD = standard deviation, <5% = difference between the means is significant at 5% level of random error.

Table 3. Descriptive and comparative statistics for body mass (kg).

Group	Chinese		Cypriot		Egyptian		Hungarian		
Age	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P
10	30.15	5.42	31.89	4.66	32.01	6.46	34.32	5.23	<5%
11	35.38	6.81	37.50	5.92	35.81	7.35	40.87	5.41	<5%
12	40.04	6.59	42.00	6.68	38.77	7.40	45.96	7.43	<5%
13	43.81	7.38	48.13	8.75	43.76	8.66	48.49	7.53	<5%

SD = standard deviation, <5% = differences between the means is significant at 5% level of random error.

Table 4. Descriptive and comparative statistics for relative body mass (kg x cm^{-1}).

Group	Chinese		Cypriot		Egyptian		Hungarian		
Age	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P
10	22.08	3.97	23.13	3.38	23.90	4.82	24.46	3.73	<5%
11	24.92	4.80	26.08	4.12	25.44	5.22	27.83	3.68	<5%
12	27.16	4.47	28.18	4.48	26.49	5.06	30.15	4.87	<5%
13	28.44	4.79	30.49	5.54	28.98	5.73	30.68	5.03	<5%

SD = standard deviation, <5% = difference between the means is significant at 5% level of random error.

Table 5. Descriptive and comparative statistics for metric index (cm).

Group	Chinese		Cypriot		Egyptian		Hungarian		
Age	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P
10	-1.41	0.27	-1.32	0.23	-1.38	0.30	-1.58	0.32	<5%
11	-1.46	0.28	-1.39	0.24	-1.43	0.31	-1.63	0.31	<5%
12	-1.52	0.34	-1.45	0.26	-1.55	0.30	-1.69	0.34	<5%
13	-1.50	0.29	-1.43	0.30	-1.53	0.32	-1.72	0.32	<5%

SD = standard deviation, <5% = difference between the means is significant at 5% level of random error.

standard deviations were similar in the four nations, and no mean dependent variabilities were found. The decreasing rank order of mean height is Hungarian, Cypriot, Chinese and Egyptian in all the four age groups.

Differences between the body mass means were also significant. The Hungarian children were the heaviest consistently considering the absolute and relative meanings as well (Table 3 and 4). The average body masses of the Chinese, Cypriot, and Egyptian boys were very similar. The only exception was the significantly greater mean of the 13-year-old boys (48.1 vs. 43.8 kg).

Tables 5 and 6 contain the means and standard deviations of growth type indices. The Hungarian boys could be characterised by the most negative metric index means from all the four age groups. However, the most linear (leptomorph) body built refers to greater bone-muscle development that is indicated by the plastic index means. By the increasing linearity component (metric index) of the growth type the rank order is: Hungarian, Chinese, Egyptian and Cypriot. It does not coincide with the trend that has been represented by the height means. The differences between the average growth type indices of Chinese and Egyptian boys were not significant at 5% level of random error in these samples.

Excluding the significant height differences from the variability of plastic index (Table 7), the consistent differences mentioned earlier disappear. At last the 10-year-old Egyptian boys and the 12-year-old Chinese adolescents had significantly greater relative plastic index than those of the remaining three averages in the respective age groups.

Another interesting observation is the timing of peak linearity (the most negative metric index within one nation). The most negative metric index means were found at 12 years of age in the Chinese, Cypriot and Egyptian samples, and the average metric indices have become greater (less negative) consistently at the 13-year-old boys. The mean trend described by the growth type indices of the investigated Hungarian subjects has indicated a significant linear decrease in accordance with the earlier published data (Szmodis et al. 1976; Mészáros et al. 1981; Mészáros and Mohácsi 1987).

By the cardiorespiratory endurance (estimated by the running time of a 1,200 m distance) our subjects can be divided in two groups (Table 8). Significantly better performances refer to the Cypriot and Hungarian subjects, and the longer times (slower running speed) to the Chinese and Egyptian samples, though the standard deviations around the means were very similar. By the evaluation of PE teachers

Table 6. Descriptive and comparative statistics for plastic index (cm).

Group	Chinese		Cypriot		Egyptian		Hungarian		
Age	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P
10	63.18	2.94	65.03	3.59	65.21	5.31	66.69	3.73	<5%
11	66.29	4.32	67.99	3.55	67.03	4.36	68.31	3.89	<5%
12	70.78	4.76	70.24	4.29	69.01	4.26	70.97	3.73	<5%
13	72.91	4.42	73.77	5.18	71.49	5.56	74.18	4.53	<5%

SD = standard deviation, <5% = difference between the means is significant at 5% level of random error.

Table 7. Descriptive and comparative statistics for relative plastic index.

Group	Chinese		Cypriot		Egyptian		Hungarian		
Age	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P
10	46.28	2.15	47.15	2.60	48.69	3.96	47.53	2.40	<5%
11	46.69	3.04	47.28	2.47	47.62	3.09	46.51	2.65	NS
12	48.03	3.23	47.12	2.88	47.15	2.91	46.56	2.45	<5%
13	47.32	2.87	46.98	3.28	47.34	3.68	46.94	2.87	NS

SD = standard deviation, <5% = difference between the means is significant at 5% level of random error, NS = difference between the means is not significant.

Table 8. Descriptive and comparative statistics for 1200 m run (s).

Group	Chinese		Cypriot		Egyptian		Hungarian		
Age	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P
10	402.9	34.8	362.6	38.6	409.1	48.5	380.1	46.3	<5%
11	399.2	40.6	359.9	37.8	419.2	51.1	369.6	42.4	<5%
12	395.1	39.3	356.3	39.6	392.6	45.9	359.8	35.2	<5%
13	389.9	39.6	351.7	41.9	389.3	46.4	348.7	34.7	<5%

SD = standard deviation, <5% = difference between the means is significant at 5% level of random error.

the above mentioned weaker performances cannot be qualified as real running.

Discussion

The simplest explanation would be that the observed differences have arisen from the various ethnicity. Though the investigated subjects are living in three continents in different geographical and cultural regions, this explanation alone is rather stuff.

The lifestyle, elementary habits and habitual physical activity strongly correlate with body mass (especially with relative body mass) and cardiorespiratory endurance, but these environmental effects do not influence on stature and physique, if the biologically necessary nutrients are continuously consumed. Though our subjects were not fat or obese (one of the grouping criteria was the body fat content being less than 25%), they cannot be qualified as lean pupils. The basic function of physical education in Egypt and Malaysia is to ensure some physical activity between the theoretical classes. The improvement of different basic abilities like endurance, speed, strength, etc., and motor learning does not belong to its tasks.

For the consistent height and physique differences we only have theoretical explanations. Anthropologically, the two European samples are closer to each other than the Egyptian to the Chinese. Consequently, or accidentally, the Europids were the tallest, however, the mean differences between the metric index means of the Cypriot and Hungarian samples were the largest. In spite of the similar height their body proportions and physique were significantly different. The Cypriot society is a relatively closed one with a short breeding radius (the Turkish-Greek marriage was exceptional), and following the civil war this isolation has increased. In Hungary the immigration, the effects of heterosis seem to be continuous during the past centuries. The more linear body built and the taller stature could be related to these effects.

The morphological differences between the Chinese and Arabic samples were very moderate in spite of the more than 11 thousand km geographic distance, since they are closest to the Equator. In our opinion the marked differences would be more obvious than the similarities, nevertheless, both the Malaysian-Chinese and the Egyptian communities are biologically more closed (religion and traditions) than the

Cypriot society. The respective results could be evaluated as sampling error, since about 100 children per age groups were investigated, but this frequency was enough to ensure the morphological variability in the other two possible comparisons. Excluding the possible effects of sampling, no definitive explanations could be given for the observed similarities.

The break point in the age-related trend of metric index relates to the time of biological maturation (Szmodis et al. 1976). The earlier break point (at 12 years of age) of the Chinese, Cypriot and Egyptian boys may indicate their earlier maturation. Since there are close similarities in the socio-economic position (consequently, the living standards of our subjects), pediatric care, caloric intake (only the amount, but not the quality of nutrients) and habitual physical activity, etc., of our subjects, the most obvious explanation is the relationship between the timing of maturations and the ratio of sunny hours, which is markedly greater in Cyprus, Egypt and Malaysia than in Hungary.

In conclusion, the metric and plastic indices (Conrad 1963) sensitively reveal the slight differences in body built and physique resulting from ethnic differences, various physical activity, etc.

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SYMPOSIUM

Body shape of patients with chromosomal aberration*

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ABSTRACT The body shape of ten patients (4 with different forms of mucopolisaccharidosis, 2 with Cockayne syndrome and 1-1 with Klippel-Feil, Sturge-Weber, Rubinstein-Taybi and Cri du chat snydrome) are described in this study. Our aim is to call the attentiton to the importance of the detailed antropological examination of these patients, because this is one of the possible ways to understand how different genes influence of the shape of the body.
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KEY WORDS

chromosomal aberration
syndromes
body shape
proportionality

One of the ways to understand the genetic effects on the body shape and the growth is to study the body dimensions in monogene disorders and chromosomal disorders. The number of such publications is limited because of two difficulties. First is the rarity of these syndromes: it is practically impossible to gather enough data for statistical analysis. The other difficulty in this work is that there is no generally used anthropometrical protocol avail-able. The description of the syndromes in the literature used contains some body measurements of the given subject, but these are not enough for a detailed analysis of the body shape. Mainly general remarks on the body shape are given as "backwardness of growth" or "craniofacial dysmorphy".

The influence of the X and Y chromosomes (Ross et al. 1983; Varrela 1984; Varella and Alvesalo 1984; Eiben et al. 1985) and also the chromosome 21– at least the distal part – on the growth and body shape (Buday 1990) are better known because the different anomalies of the sex chromosomes, and also the trisomy and the translocation forms of the Down's syndrome, are more frequent.

Some of these syndromes involve normal intelligence but mental retardation is found among the features most of the time. There are more than 500 genes which are able to cause mental retardation. Their effects on the body shape are not known.

Materials and Methods

We present the body shape of ten patients living in different institutions and nursing homes in Hungary. Some of these syndromes are metabolic disorders, caused by one gene. It means that the product of this gene is missing from a metabolic chain. Therefore, the given chain changes its direction and the by-products have an influence on the brain and/or the mental capacity, and also on some other obligatory or facultative features. So it has pleiotropic effects including an influence on the growth and the body shape because of the disturbed metabolic chain. We have some other patients with structural disorders of different chromosomes.

In the following descriptions, some characteristic features are summarized, mainly which concern the growth and body shape. Then, the proportionality profile is shown. The somatotype components were also calculated for all subjects with the aim of studying the physique. The distance between the individual somatoplots and the control ones of the same age and sex, the somatotype attitudinal distance were calculated according to Duquet and Hebbelinck (1977). The control data were calculated from the Nationwide Growth Study (Eiben et al. 1991).

Results and Discussion

Mucopolysaccharidoses

This is a group of diseases called lysosomal storage disorders. It has ten different forms and subforms due to the lack of different enzymes in the metabolism of glycosaminoglycans. We have four cases in this syndrome.

Patients 1 and 2 on MPS IV. (Maroteaux-Lamy syndrome)

Backwardness of the growth, craniofacial dysmorphy, convex sternum, lumbal kyphosis and scoliosis. Hard of hearing. Recessive inheritance. Locus D:12q14, McKusick number is 252900. The most frequent form of MPS, frequency is: 1:25,000.

Our patients are a 10-year-old dizygotic twin pair. Almost all of our boy patients' body measurements are proportionally lower than that of the control (Fig. 1). There are some exceptions: length of the extremities and the humerus and femur width. The proportion of the girl pair is similar (Fig.

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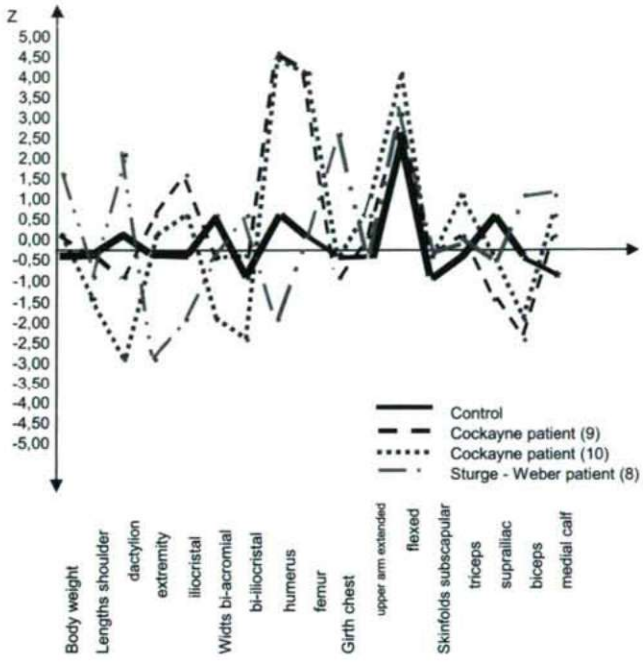


Figure 1. Proportionality profile of 10-year-old boy patients.

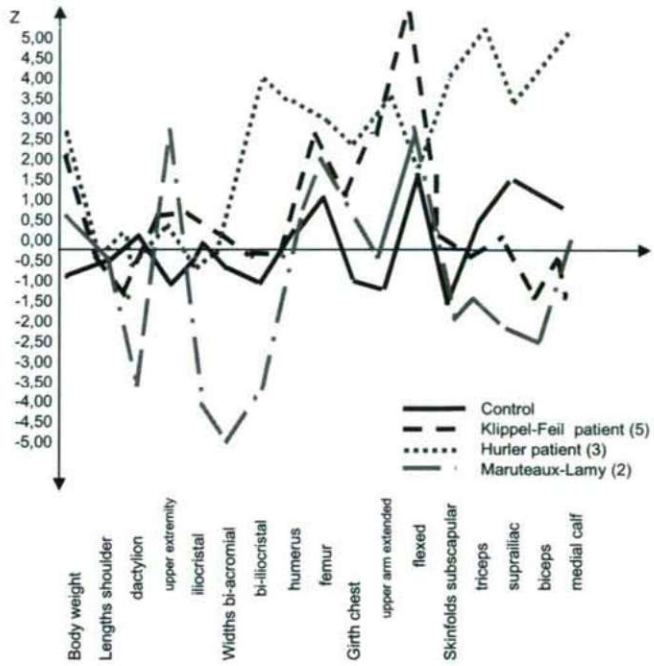


Figure 2. Proportionality profile of 10-year-old girl patients.

2) but the length of the lower extremity is proportionally lower than that of the control.

Patient 3. MPS I. (Hurler syndrome)

In this form, the α-I-iduronidase enzyme is absent. Mental retardation and hearing impairment due to the quantity of ganglioside increase in the nervous system. Imbecillity. The "gargoil" face, short stature and deformity of skeleton (thoracolumbal gibbus, pectus carinatum, macrocephalia) are characteristic. Locus: 4p16.3, McKusick number is 252800, inidence: 1:25,000.

We have a 10-year-old girl, her proportionality profile is shown in Figure 2. The weight, girths, skinfolds and the biepicondylar width are proportionally higher than that of the controls of the same gender and age.

Patient 4. MPS III. (Sanfilippo syndrome)

Sanfilippo is the most frequent form of the MPS, frequency is 1:25,000. There are four different subforms in this syndrome. Growth is retarded, the features often characterized only after the second year of life. Head girth is higher than the normal probably due to the thick cranial bones. Recessive inheritance.

Our case is a Sanfilippo A form, which means the lack of heparan-N-sulfatase enzyme. McKusick number is 252900.

He is 10 years old and his lengths and widths and all skinfolds are proportionally lower than that of the control of the same age (Fig. 1).

Patient 5. (Klippel-Feil syndrome)

Main features are craniofacial dysmorphy, short neck with limitation of motion due to the fusion of cervical vertebrae and atlas assimilation. Frequent association with other syndromes. Autosomal dominant inheritance.

Incidence is 1:50,000, more in females. Locus is not known. McKusick number is 148900.

Our patient is a 10-year-old girl (Fig. 2). Her lengths and the biacromial and iliocristal widths are proportionally less than that of the controls of the same gender and age. The humerus and femur widths and the girths are somewhat higher than that of the control but the skinfolds are lower. There are significant differences in the body proportion of this girl and the two other cases with mucopolysaccharidosis.

Patient 6. (Cri du chat syndrome)

The main feature is that the sound of the crying of these children is similar to the cat mew, because of the developmental disorder of the laryngs. Microcephaly, hypertelorism, lip and/or cleft palate.

This is a deletio: the 5p15 monosomy. Most of the cases are de novo deletio. Prevalency at birth is 1/50,000 but the frequency among mentally retarded is 1.5/5,000. Male-female ratio is 1:4.

Our patient is a 7-year-old boy. Weight, length of his extremities, the condylus width and the girths are proportionally higher than that of the control of the same sex and age (Fig. 1). There are also significant differences betwen the

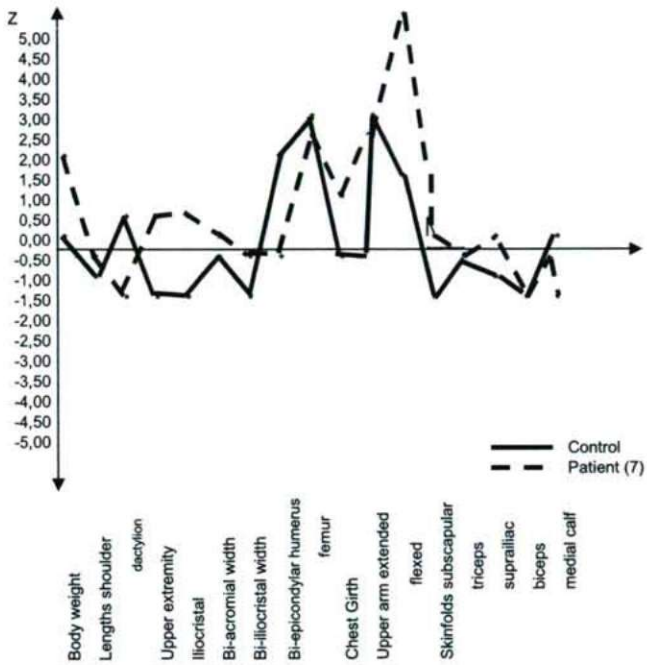


Figure 3. Proportionality profile of 5-year-old female patient with Rubinstein-Taybi syndrome.

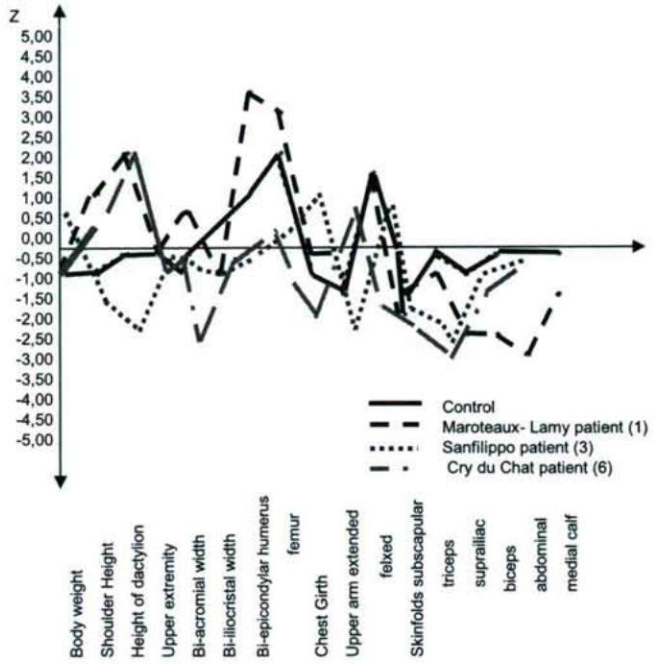


Figure 4. Proportionality profile of patients No 8, 9 and 10.

proportion of this boy and the two other same-age patients with mucopolysaccharidosis.

Patient 7. (Rubistein-Taybi syndrome)

Main features of this syndrome are the short stature, craniofacial dysmorphy with beak-like nose, hypertelorism, microcephaly, epicantus, maxillar hypoplasia, gothic palate, characteristic deformation of the fingers and toes (brachymegalophalangia). Imbecillity. Facultative micrognatia, pectus excavatum or carinatum, skeletal age is delayed. Autosomal dominant inheritance, locus: 16p13.3, McKusick number: 180849. Not rare.

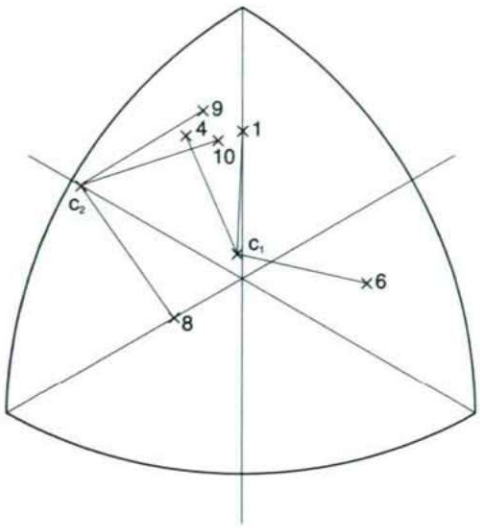
Our patient is a 5-year-old girl (Fig. 3). Her extremities and also the biacromial and bi-iliocristal width are proportionally longer than that of the control.

Patient 8. (Sturge-Weber syndrome)

This syndrome is characterized by the cutaneous angiomatosis which is unilateral on the area of nervus trigeminus and sometimes of the neck, trunk and extremities. Imbecility and focal epilepsy due to the haemangiomatosis extended to the leptomenings. Contralateral hemiparesis is frequent. Retardation of the growth. Mendelian inheritance is not proven although some cases with dominant inheritance were de-

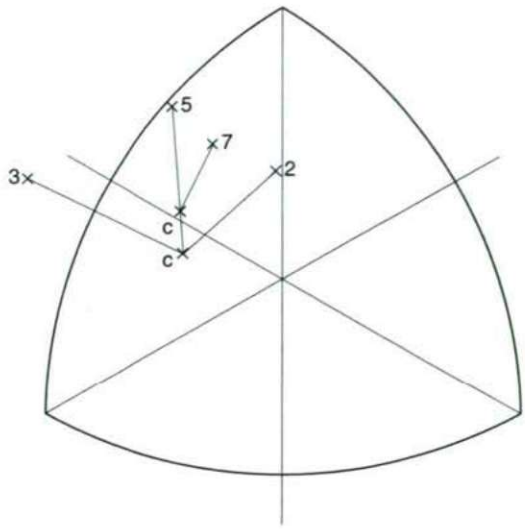
Table 1. Somatotype components of the patients.

Number	Age	Sex	I II III	SAD
7.	5	Female	2.00- 4.00- 0.50	2.79
Control			3.99- 4.98- 2.19	
1.	10	Male	1.00- 5.00- 1.00	3.83
4.	10	Male	3.00- 5.50- 0.50	2.79
6.	10	Male	1.00- 2.50- 4.00	3.15
Control			3.67- 4.17- 3.61	
2.	10	Female	1.50- 4.00- 1.50	4.25
3.	10	Female	7.00- 6.00- 0.50	4.58
5.	10	Female	3.50- 5.50- 0.50	4.15
Control			5.03- 3.56- 3.84	
8.	19	Male	2.00- 2.00- 4.00	5.44
9.	18	Male	2.50- 5.50- 1.50	2.85
10.	21	Male	2.50- 5.50- 2.00	2.93
Control			5.33- 5.30- 1.27	



C_1: Control for 10-year-old patients, C_2: Control for 18-year-old patients.

Figure 5. Somatochart for boy patients.



C_1: Control for 5-year-old patients, C_2: Control for 10-year-old patients.

Figure 6. Somatochart for girl patients.

scribed. McKusich number is 185300. Frequent. Our case is a 18-year-old boy. His extremities and widths are proportionally lower than that of the control (Fig. 4).

Patient 9 and 10. (Cockayne syndrome)

Loss of adipose tissue is characteristic to this syndrome from early infantile period. Craniofacial dysmorphy. Rapid ageing. Microcephaly, expressed dorsal kyphosis, big hands and feet.

Autosomal recessive inheritance. Locus is not known, McKusick number is 216400. Frequency is not known.

Our patients are two elder brothers from a four-sibling family. They have a younger sister and a brother, none of them are affected. Our younger patient (9) is 18, the older one (10) is 21 years old. Their proportions are similar: the extremities and biacromial and iliocristal widths and most of the skinfolds are lower than that of the controls but it is remarkable that the humerus and femur widths are proportionally larger. This is probably in connection with the rapid ageing, which is one of the obligatory signs of this syndrome.

The somatotype components of the patients are shown in Table 1. They were grouped according to their age and sex. The components of the corresponding control groups are also shown. The individual somatoplots are compared with the control for the boys on the Figure 5 and for the girls on Figure 6. It is remarkable that the highest values of the SAD – therefore the greatest distances from the control – are in the cases with mucopolysaccharidosis. It is also interesting that the proportionality profiles of these patients are similar to each other but differ from other cases. Great distance from the control somatoplots in the Sturge-Weber case was also found.

Our intention was to call the attention to the importance of this kind of examinations. The presented cases are not enough to give correct conclusions on the mentioned gene effect on the body shape. It is important to collect the similar cases.

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SYMPOSIUM

Supernumerary occlusal cusps on permanent human teeth+

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ABSTRACT Supernumerary (central) cusps that appear on the occlusal surface of the teeth have already been grouped by many authors. The most comprehensive grouping of central cusps on the premolars is that by Schulze (1987). However, different central cusp forms may occur on the molar teeth, and cusp-like protrusions may also develop on the incisors and the canines. In the present work, plaster casts of the teeth of 500 orthodontic patients (250 males and 250 females) were examined for central cusps at the above departments. The central cusps appeared on the dentition of 47.6% of the investigated Hungarian population. From a total of 13,793 teeth examined, 501 (3.69%) were observed to display a central cusp. The teeth most often affected were the mandibular first premolars (11.6%). A new central cusp form, the "margoid central cusp formation," was noted. **Acta Biol Szeged 46(1-2):71-82 (2002)**

KEY WORDS

central occlusal cusps
premolars
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incisors
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morphological characteristics
population relationship

Central cusps (occlusal supernumerary cusps) are situated between the buccal and lingual cusp tips on the occlusal surface of the premolars and molars, and on the lingual surface of the incisors and canines. The first description was provided by Leigh (1925), who reported an enamel tubercle on the third maxillary right molar of an Eskimo skull. Central cusps have subsequently been described by many authors (Jyojima 1929; Yumikura and Yoshida 1936; Lau 1955; Oehlers 1956; Allwright 1958; Merrill 1964), though in different forms, on premolar, molar, incisor and canine teeth.

Some authors classify an enlarged tubercle on the lingual surface (talon cusp) of the incisors among occlusal central cusps; this is known as the dens evaginatus (Shey and Eitel 1983; Dankner et al. 1996a; Uyeno and Lugo 1996). The different forms of central cusps were grouped on the basis of their location and shapes by Lau (1955), Merrill (1964) and Schulze (1987), with special regard to the premolars.

From an anthropological point of view, the significance of the study of these cusps is that their frequencies differ from population to population. Recognition of the cusps is also important from the aspect of clinical dentistry, because they may be associated with other anomalies, and these phenomena can lead to pathological complications.

Our goal is to review the literature relating to occlusal supernumerary (central) cusps on the different tooth types, and to report data concerning these cusps in a Hungarian sample.

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+Dedicated to Professor Gyula Farkas on the occasion of his 70th birthday.

Forms of occlusal central cusps

Premolars

Most of the forms of occlusal cusps can be observed on premolar teeth. Lau (1955) distinguished two groups: cusps grown out of buccal cusps, and cusps grown out of the middle of the occlusal surface. They can be smooth, grooved, terraced or ridged. Merrill (1964) slightly modified Lau's classification of the second group to include a double lingual cusp as a sub-group, although he had observed it on a lower tooth in one case only. Yip (1974) and Schulze (1987) grouped the Lau and Merrill types into one class, named the dens evaginatus.

Schulze (1987) concluded that central occlusal cusps are mainly characteristic of east Asian populations and that they rarely occur in other populations. He distinguished five different shapes on the premolars. The first and second shapes were described by Schulze himself, whereas the other three shapes were taken from the earlier literature. The shapes are as follows:

1) A cone-like enlargement of the lingual cusp. This is a gradually growing serial characteristic, with the following phases:

a) A cone-shaped lingual cusp more distinct in the buccal direction;

b) A significantly enlarged lingual cusp with distinct marginal wrinkles;

c) A separately developed central cusp on the lingual crown side. The marginal wrinkles merge into a cingulum.

2) A supernumerary cusp, which is similar to the previous (c) shape. The original lingual cusp is clearly seen next to the central cusp. We have re-evaluated a case reported earlier (Kocsis, 1984) and included it in this group: a supernumerary

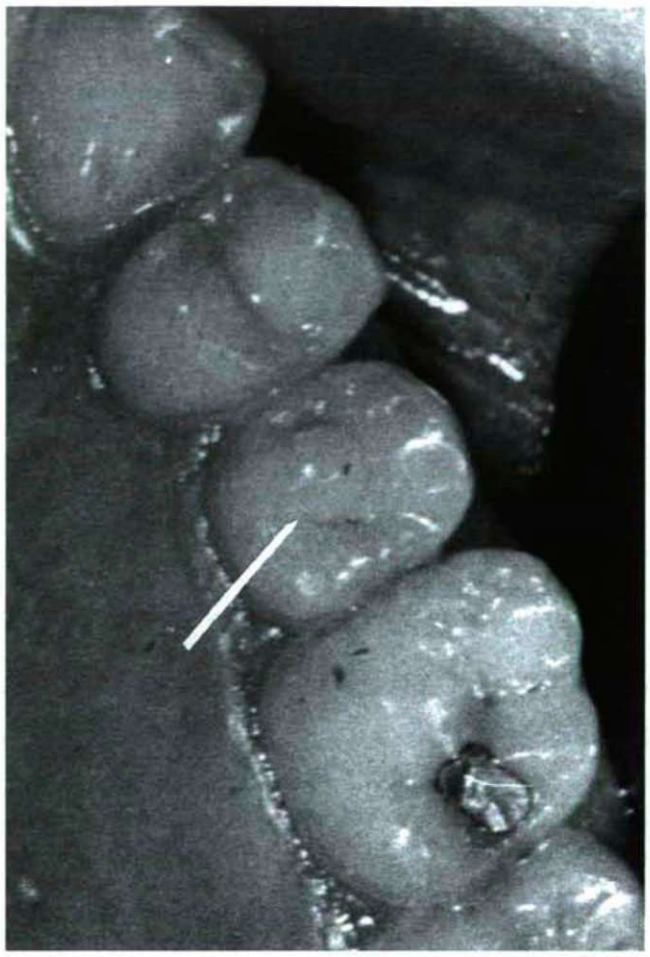

Figure 1. Supernumerary occlusal cusp on the lingual side of the maxillary left second premolar of a Hungarian girl. The central cusp is larger than the normal cusp.

Figure 2. Dens evaginatus on the lower second premolar of a Vietnamese girl. The central cusp is worn, a dentinal island can be seen.

occlusal cusp on the maxillary left second premolar (Fig. 1) of a 12-year-old Hungarian girl. There is no familial history of this trait.

3) The dens evaginatus is the best-known central cusp. It is an extra cusp, a form of a tuberculum arising from the occlusal surface. The term dens evaginatus was recommended by Yip (1974) and it is the most frequently used term today; however, there are other terms: premolar odontomes, occlusal tubercles, or tuberculated premolars (see Scott and Turner 1997). Such occlusal cusp formations were first described by Japanese authors (Jyojima 1929; Matsumura 1934; Yumikura and Yoshida 1936). Kato (1937) reported that they were present in 1.09% of the Japanese population. The types and locations of the dens evaginatus are known from the publications of Lau (1955) and Merrill (1964).

The dens evaginatus appears in different populations of East Asian ancestry, with a frequency of 0.5-4.3% (Table 1). The term "Mongoloid or oriental premolar" reflects its occurrence; it appears with different frequencies in Japanese, Chinese, Malayan, Eskimo, American Indian and Thai populations (Curzon et al. 1970; Reichart and Tantiniran 1975). A dens evaginatus was found on the premolar of a Vietnamese patient treated in Hungary (Fig. 2). Several authors have described evagination on premolars in Filipinos (Villa 1956; Villa et al. 1959; Poyton and Vizcarra 1965; Senia and Regezi 1974).

As regards Sub-Saharan Africans, the literature mentions only 2 cases (Table 1). In one of these, a dens evaginatus was found by Pearlman and Curzon (1977) on the second left mandibular premolar of a male, while the other case was discovered by Ciechanowski and Sonnenberg (1981), on both mandibular first premolars and the second right premolar of a female. In the latter case, one of the female's great-grandmothers was partly Cherokee Indian. The teeth of her parents and siblings showed no evidence of this anomaly.

The above form of the dens evaginatus occurs rarely in Europeans (Table 1). Palmer (1973) described it in 4 British males and considered that the accentuated lingual aspect of

Table 1. The prevalence of dens evaginatus in different populations.

Author(s)	Year	Population	No. of affected persons	%
Yumikura and Yoshida	1936	Japanese	17	-
Kato	1937	Japanese	-	1.09
Pedersen	1949	Eskimo (Greenland)	5	0.50
Lau	1955	Chinese	27	1.29
Wu	1955	Chinese	19	1.44
		Chinese	16	1.52
Oehlers	1956	Malays	110	-
Sumiya	1959	Japanese	-	1.88
Merrill	1964	Eskimo (Amerindian)	28	4.30
Oehlers et al.	1967	Chinese (Malays)	43	-
Curzon et al.	1970	Eskimo (Canada)	12	3.00
Yip	1974	Chinese	21	3.60
		Malay	3	1.10
		Indo-europoid	-	-
Reichart and Tantiniran	1975	Thai	51	1.01
Goto et al.	1979	Japanese	53	0.12
Lin and Roan	1980	Chinese (Taiwan)	305	3.52
Villa	1956	Filipino	1	-
Villa et al.	1959	Filipino	2	-
Poyton and Vizcarra	1965	Filipino	1	-
Senia and Regezi	1974	Filipino	1	-
Palmer	1973	Caucasian (British)	5	-
Sykaras	1974	Caucasian (Greek)	1	-
Pearlman and Curzon	1977	Negro	1	-
Ciechanowski and Sonnenberg	1981	Negroid	1	-

the buccal cusp on the premolars of the sister of one of the males was also most probably a dens evaginatus. Sykaras (1974) observed this anomaly on the premolars of a Greek female.

Evagination can be associated with other developmental anomalies: invagination, an extra premolar and mesiodens (Yip 1974; Geist 1989) and three-rooted mandibular molars (Senia and Regezi 1974).

The clinical significance of this disorder is that the elevation sooner or later becomes damaged, breaks off or wears away during use of the tooth. In a large majority of the cases, the root canal opens and the pulp chamber becomes infected. As a result of the malocclusion of the teeth, complications can develop: irregular development of the root, or the tooth becomes irregularly positioned and loosens (Allwright 1958; Reichart and Tantiniran 1975; Goto et al. 1979).

4) An extra occlusal cusp is situated on the lingual surface of the buccal cusp. It resembles an enamel pearl. Nishijima et al. (1959) described it in Japanese as "several cases of central tubercle on the lingual ridge of buccal cusp of upper bicuspid" (p. 1209). In all probability, the same phenomenon was described by Pedersen (1949), who reported an occlusal pearl on a maxillary second premolar, in 2 cases unilaterally on mandibular second premolars, and bilaterally on a second and a first premolar pair. He stated that "in the East Greenland Eskimo dentition we meet with anatomical features the significance of which, if any, is obscure, ... the occlusal pearl-like excrescences" (p. 214).

5) Kirveskari et al. (1972) described bulging of the lingual aspect of the buccal cusps in Lapps. This was observed on the lingual ridge of the buccal cusps of premolars, and also on the mesiobuccal cusp of molars. It occurs symmetrically and seems to be more frequent on the maxillary teeth and also on the second premolars and first molars. The dentin does not show this bulge. They presumed that it occurs widely in Northern populations. Schulze (1987) also considered it to be population-specific. The same type of central cusp was found by Kutscha (1985) on a premolar in a German population sample. It seems likely that the accentuated lingual ridge of the buccal cusp on the premolars of a British girl mentioned by Palmer (1973) also belongs here.

Marcsik and Kocsis (1986) carried out a survey of 31 skulls from the 8th century (A.D.) in Hungary that encompassed 79 upper and 58 lower premolars. (The data on central cusps are unpublished.) The above-described form of central cusps was found on 7 premolars (5.11%) (Fig. 3).

Molars

The central cusps on the occlusal surface of molar teeth have also been classified as dens evaginatus (Lau 1955; Merrill 1964; Oehlers et al. 1967).

A central tubercle was described by Pedersen (1949) as "a peculiar enamel pearl-like 'cusp' on the occlusal surface" (p. 85) on the right upper molar of an Eskimo from Greenland. In his opinion, the case of an American Eskimo reported by Leigh (1925) had the same form.

Figure 3. Bulging of the lingual surface of the buccal cusp of a lower right second premolar. Archeological material, 8th century. Juvenile specimen.

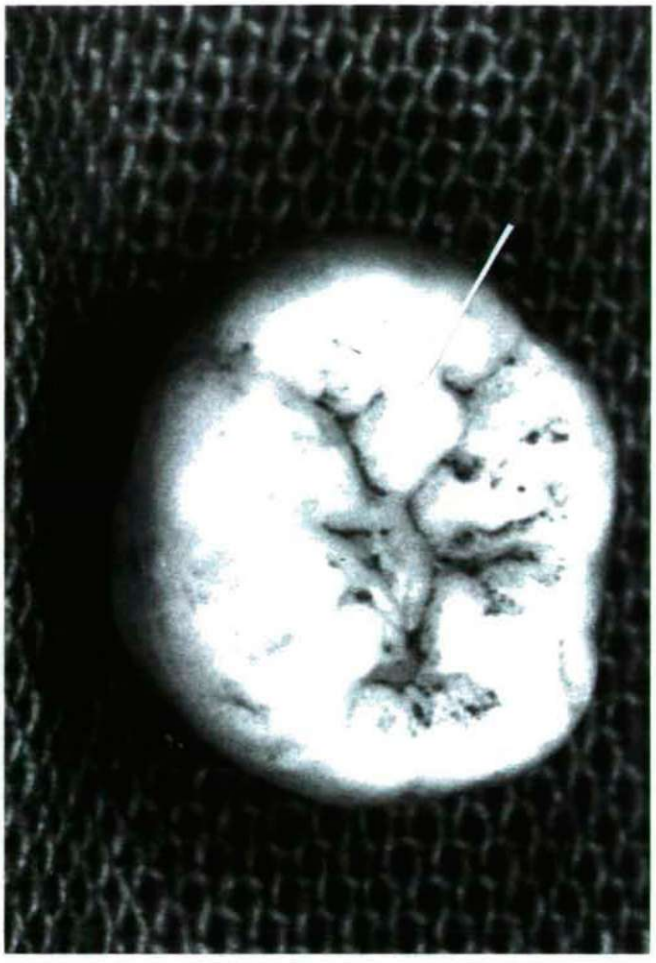

Figure 4. Occlusal enamel pearl on the lower right second molar. Archeological material, 8th century. Juvenile specimen.

Kirveskari et al. (1972) analysed bulging of the lingual aspect of buccal cusps on molars, similar to that on premolars. The third molar was the least affected, and the maxillary first molar and the second premolar were the most commonly affected.

Marcsik and Kocsis (1986) examined – from the above mentioned material – 106 upper and 86 lower molars of the 31 skulls, and found that bulging of the lingual aspect of the buccal cusps occurred on 8 maxillary molars (4.16%). Occlusal enamel pearl was found on 2 lower molars (Fig. 4).

Incisors and canines

The dens evaginatus occurs in a similar way on canines and even on incisors; as a result of the vulnerability of the elevation, it has the same clinical significance (Lau 1955; Oehlers 1956; Allwright 1958; Merrill 1964; Goto et al. 1979).

The talon cusp was recently reported as a subclass of the dens evaginatus (Dankner et al. 1996a, b; Uyeno and Lugo 1996), and as an accessory cusp on the lingual (rarely on the buccal; McNamara 1997) surface of the anterior teeth. The first recorded case of a talon cusp originates from 1892, when Mitchell described it as "a process of hornlike shape, curving from the base downward to the cutting edge". It may form a connection with the incisal edge to produce a T-form or a Y-shaped crown contour (de Jonge 1959; Hattab et al. 1996). This form is referred to in the dental anthropological literature as the "triform variant" (Bailey-Schmidt 1995). If the anomaly appears with occlusion, it has similar clinical significance to that of the dens evaginatus (Uyeno and Lugo 1996). The reported prevalence of talon cusp ranges from 0.06% to 7.7% (Chawla et al. 1983; Sedano et al. 1989). An investigation of 1997 skulls ranging in origin from the Neolithic to the Middle Ages in Hungary revealed anomalies on anterior teeth (Kocsis 1994). In this study the overall

prevalence of lingual tubercles among all of the evaluated maxillary teeth (n=6,383) was 2.41%, and the lingual cusps, the talon cusp form occurred on 2 maxillary lateral incisors.

This cusp can arise on primary anterior teeth, too (Henderson 1977; Mader and Kellogg 1985; Chen and Chen 1986; Meon 1990; Rusmah 1991). It can be associated with other dental disorders, such as agenesis, peg-shape, supernumerary teeth, impaction, shovel-shape, bifid cingulum, the dens invaginatus, a labial groove, an accessory cusp or an accessory root on other teeth and the dens evaginatus of posterior teeth (Mader 1981; Natkin et al. 1983; Davis and Brook 1985; Acs 1992; Hattab et al. 1996). The etiology of the talon cusp is unknown. As it occurs with other anomalies of tooth number and size, it may have a multifactorial etiology, involving both genetic and environmental factors (Davis and Brook 1985), which accounts for its occurrence with other features.

Pedersen (1949, p.174) recorded 6 upper canines with enamel pearl on the "inciso-lingual" surface in 4 Eskimo dentitions. They seem to be similar to the cases reported on the anterior teeth by Lau (1955), Allwright (1958) and Goto et al. (1979).

Kirveskari et al. (1972) described bulging of the lingual aspect of the buccal cusps on premolars and molars, which is sometimes found on the lingual aspect of the canine cusp tip, too.

Central cusps in syndromes

A syndromic characteristic is the occurrence of central cusps on both premolars and molars in lobodontia (Robbins and Keene 1964; Shuff 1972; Schulze 1976; Brook and Winder 1979) in an unusual triad: microdontia, taurodontia and dens invaginatus (Casamassimo et al. 1978), and in multiple anomalies of teeth which are similar to lobodontia but differ from it in several ways (Ekman-Westborg and Julin 1974; Reichart and Triadan 1977; Reichart et al. 1978; Miikada et al. 1995; Ritzau et al. 1997, Yoda et al. 1998). Another case was described in a 5-year-old child from the 15th century as a variant of the Ekman-Westborg-Julin syndrome, but dens evaginatus is not characteristic (Mann et al. 1990).

A case of lobodontia was also reported by Kocsis et al. (1994). In the permanent dentition of a 16-year-old boy, the anomaly affected practically all the teeth (irregular tooth form, hypodontia, delayed tooth eruption and diminished tooth size). Since this publication, the patient's lower left third molar has erupted and has now been examined. On the occlusal surface of the tooth, a large central cusp can be observed, surrounded by more than 10 smaller cusps. The large occlusal cusp is separated from the smaller cusps by a deep groove (Fig. 5).

The talon cusp – dens evaginatus on anterior teeth – appears to be more prevalent in the Rubinstein-Taybi syndrome (Gardner and Girgis 1979), the Mohr syndrome (Goldstein and Medina 1974), the Sturge-Weber syndrome (Davis and Brook 1985) or incontinentia pigmenti acromians (Tsutsumi and Oguchi 1991).

Figure 5. A large central cusp on the lower left third molar of a patient with lobodontia.

The purpose of this study is twofold: 1) determination of the association of cusp types with each other and with specific teeth; 2) identifiable cusp types (those statistically identified) with sex.

Materials and Methods

A total of 500 plaster casts of dentitions were at the authors' disposal for central cusp examination. These study models had been produced at the University of Szeged, from impressions taken from orthodontic patients aged between 12 and 23 years (mean age 14.7 years) in 1997. The ratio of males and females was 1:1.

All of the 500 models used in the present study met the following conditions: all of the permanent teeth (excluding third molars) were present in the dentition to permit con-

Figure 6. Cone-shaped lingual cusp enlarged in vestibular direction on a lower right second premolar.

Figure 8. Bulging of the lingual surface of a buccal cusp of a lower second premolar in the investigated sample.

Figure 7. Talon cusp. Marked abnormal cusp on lingual surface of a permanent maxillary central incisor.

firmation of the identity of the teeth, and relevant crown areas (see later) were completely or almost completely intact.

The number of teeth suitable for investigation was less than the number of models could have allowed because third molars, unerupted teeth and filled teeth were excluded. Thus 6,897 teeth for males and 6,896 teeth for females constituted the sample.

On the basis of our literature review, we define central cusp as a supernumerary macrostructure of the tooth surface, representing a cusp formation on the occlusal surface of premolars and molars, and on the lingual surface of canines and incisor teeth. The macrostructure of a central cusp involves not only the strengthening of the original enamel layer of a cusp, but also the presence of a circumscribed, well-defined elevation. The surrounding cusp enamel may sometimes be separated from the cusp by a mild groove. The types of central cusps can be characterized as given below.

Type 1) Registration surface: occlusal. Enlargement or bulging on the buccal surface of a lingual cusp of premolars

Table 2. Frequency of central cusps in a Hungarian sample.

		Males teeth	Females teeth	Total teeth
Lingual conical cusp (type 1)				
	premolars	1,984	1,986	3,970
	with cusp	22(1.1%)	42(2.11%)	64(1.61%)
	molars	1,940	1,931	3,871
	with cusp	12(0.61%)	25(1.29%)	37(0.95%)
	total posterior teeth	3,924	3,917	7,841
	with cusp	34(0.87%)	67(1.71%)	101(1.29%)
Separated lingual cusp (type 2)				
	posterior teeth	3,924	3,917	7,841
	with cusp	5(0.13%)	1(0.03%)	6(0.08%)
Buccal bulging (type 5)				
	all of teeth	6,897	6,896	13,793
	with cusp	141(2.04%)	204(2.96%)	345(2.5%)
Lingual tuberculum (type 6)				
	max. anterior teeth	1,476	1,483	2,959
	with cusp	9(0.6%)	8(0.53%)	17(0.57%)
Margoid (type 8)				
	posterior teeth	3,924	3,917	7,841
	with cusp	24(0.61%)	26(0.66%)	50(0.64%)

and molars in the faciolingual direction. The lingual cusp is cone-shaped (Fig. 6).

Type 2) Registration surface: occlusal. A separately developed macrostructure can be seen on the lingual crown side on premolars and molars. The original lingual cusp still exists, and the macrostructure can be identified as a supernumerary lobe/central cusp located close to the lingual cusp (Fig. 1).

Type 3) Registration surface: occlusal. A supernumerary cusp on the occlusal surface arising from or near the groove between the original buccal and lingual cusps of premolars and molars. The central cusp type is the dens evaginatus (Fig. 2).

Type 4) Registration surface: occlusal on the posterior teeth, and lingual on the anterior teeth. A pearl-like enlargement is situated on the lingual surface of a buccal cusp in faciolingual direction on premolars and molars (Fig. 4). This central cusp type is the occlusal enamel pearl. It may sometimes occur on canines, too.

Type 5) Registration surface: occlusal on the posterior teeth, and lingual on the anterior teeth. Bulging of the lingual aspect of a buccal cusp on premolars (Fig. 3) and molars, and bulging of the lingual aspect of the central lobe on canines and incisors.

Type 6) Registration surface: lingual. Various degrees of supernumerary cusp formation on the lingual surface of the anterior teeth, developing from the lingual tuberculum or from the cingulum (Fig. 7).

Type 7) Registration surface: occlusal on the posterior teeth, and lingual on the anterior teeth. Occlusal (or lingual) supernumerary macrostructure of teeth in the case of syndromes (Fig. 5).

Types 8) Registration surface: occlusal on the posterior teeth, and lingual on the anterior teeth. This is a new type of central cusp not previously described in the literature.

After these types had been established, definitive registration was performed on the casts. Each individual tooth was examined with the aim of determining a positive or negative result for all types, as well as the common existence of types, and any association with other dental anomalies. The observations were made by visual inspection, and with a magnifying glass at magnification X 8, and a direct light was used.

The association of central cusp types with specific teeth were given by a relative frequency, as well as differences in the distribution of central cusp types between males and females, were determined with chi-square tests. A probability level of 1% was employed as the level of significance.

Results and Discussion

The data revealed that central cusps were present in 47.6% of these 500 Hungarian dentitions: in 148 females (59.2%) and in 90 males (36%).

Four of the forms mentioned in the literature were found in the material investigated:

1) a cone-shaped lingual cusp enlarged in the buccal direction (type 1; 101 teeth);

2) a separately developed central cusp on the lingual crown side (type 2; 6 teeth);

3) bulging of the lingual aspect of the buccal cusp (type 5; 345 teeth);

4) an enlarged lingual tubercle on the anterior teeth, including the talon cusp (type 6; 17 teeth).

Table 3. Frequency of central cusps in all tooth types (males).

Teeth	Total	7	6	5	4	3	2	1
No. upper teeth	3,431	474	487	494	500	478	498	500
Lingual conical cusp	2	-	-	2	-	-	-	-
(type 1) %	0.06	-	-	0.40	-	-	-	-
Separated lingual cusp	2	-	-	2	-	-	-	-
(type 2) %	0.06	-	-	0.40	-	-	-	-
Buccal bulging	81	12	15	30	12	12	-	-
(type 5) %	2.36	2.53	3.08	6.07	2.40	2.51	-	-
Lingual tuberculum	9	-	-	-	-	8	1	-
(type 6) %	0.26	-	-	-	-	1.67	0.20	-
(Talon cusp form)	(1)	-	-	-	-	-	(1)	-
%	0.03	-	-	-	-	-	0.20	-
Margoid	2	-	-	2	-	-	-	-
(type 8) %	0.06	-	-	0.40	-	-	-	-
No. central cusps	96	12	15	36	12	20	1	-
No. lower teeth	3,466	484	495	490	500	497	500	500
Lingual conical cusp	32	5	7	18	2	-	-	-
(type 1) %	0.92	1.03	1.41	3.67	0.40	-	-	-
Separated lingual cusp	2	-	-	-	2	-	-	-
(type 2) %	0.06	-	-	-	0.40	-	-	-
Buccal bulging	60	-	-	16	25	19	-	-
(type 5) %	1.73	-	-	3.27	5.00	3.82	-	-
Lingual tub. (type 6)	-	-	-	-	-	-	-	-
Margoid	22	-	-	1	21	-	-	-
(type 8) %	0.63	-	-	0.20	4.20	-	-	-
No. central cusps	116	5	7	35*	50**	19	-	-

*Simultaneous occurrence of buccal bulging and lingual conical cusp on same tooth in 3 cases
** Simultaneous occurrence of buccal bulging and lingual conical cusp on same tooth in 6 cases

Besides these four types of central cusps, a new cusp form was observed (type 8; 50 teeth). Data on the prevalence of these various central cusps are presented in Tables 2-4.

The most frequent type as regards all types of teeth was the bulging of the lingual aspect of the buccal cusp (type 5; Fig. 8). Its incidence was 2.5%, and it seemed to be more widespread in females (2.96%) than in males (2.04%); the difference between the sexes was significant (p=0.00059). The trait generally occurred symmetrically in the dentition, but sometimes with different expressivities on the two sides. It was most frequent on the mandibular first premolars (6.90%) and the maxillary second premolars (6.86%), followed by the maxillary second molars (4.63%). The frequencies on premolars and molars were 5.31% and 2.06%, respectively. As concerns the molars, this anomaly was more developed on the mesiobuccal cusp than on the distobuccal cusp.

In contrast with our results, Kirveskari et al. (1972) considered that this type of central cusp is more frequent on maxillary second premolars and first molars. They mentioned that this anomaly is sometimes found on the lingual aspect of the canine cusp tip. Our results demonstrated that the trait can also occur on cuspids but on incisors, too. In the 8th century sample the prevalence of lingual bulging of the buccal cusp on premolars was 5.12%, while that on molars was 4.16% (Marcsik and Kocsis 1986). These results suggest that it is a common trait in both past and present populations.

Another frequent trait was the cone-shaped form of the lingual cusp enlarged in the buccal direction (type 1) on premolars (1.61%) (Fig. 6); this central cusp form also occurred on the mesiolingual cusp of molars (0.95%). Altogether, its prevalence on posterior teeth was 1.29% (females: 1.71%, males: 0.87%); the difference between females and males was significant (p=0.00098). The lower mandibular second premolars were most commonly affected (5.2%) (Fig. 6).

Among the anterior teeth, the maxillary incisors and canines displayed enlarged (lingual) tubercles (type 6) in 0.57% of the cases. From these central cusps, a talon cusp was found in 3 cases (Fig. 7). Tables 3 and 4, relating to the maxillary anterior teeth, reveal two further prevalences: that of the enlarged lingual cusp, and (in parentheses) that of the talon cusp. It is necessary to distinguish the lingual tubercle from its talon form: the horn-like protuberance of Mitchell (1892), and the "eagle's talon" of Mellor and Ripa (1970). Hattab et al. (1996) suggested a system of lingual cusp classification based on the degree of their formation and extent (talon, semitalon and trace talon). Some authors probably reported mixed cases of the forms, and the frequency therefore presumably varies between 0.06% (Sedano et al. 1989) and 7.7% (Chawla et al. 1983). Other data indicates that the incidence of the dens evaginatus of anterior teeth may

Table 4. Frequency of central cusps in all tooth types (females).

Teeth	Total	7	6	5	4	3	2	1
No. upper teeth	3,448	477	491	497	500	484	499	500
Lingual conical cusp (type 1)	-	-	-	-	-	-	-	-
Separated lingual cusp (type 2)	-	-	-	-	-	-	-	-
Buccal bulging	128	32	20	38	29	9	-	-
(type 5) %	3.71	6.71	4.07	7.65	5.80	1.86	-	-
Lingual tuberculum	8	-	-	-	-	-	7	1
(type 6) %	0.23	-	-	-	-	-	1.40	0.20
(Talon cusp form)	(2)	-	-	-	-	-	(1)	(1)
%	0.06	-	-	-	-	-	0.20	0.20
Margoid	3	-	3	-	-	-	-	-
(type 8) %	0.09	-	0.61	-	-	-	-	-
No. central cusps		139	32	23	38	29	9	7 1
No. lower teeth	3,448	478	485	489	500	496	500	500
Lingual conical cusp	67	17	8	33	9	-	-	-
(type 1) %	1.94	3.56	1.65	6.75	1.80	-	-	-
Separated lingual cusp	2	1	-	1	-	-	-	-
(type 2) %	0.06	0.21	-	0.20	-	-	-	-
Buccal bulging	76	1	-	17	44	12	2	-
(type 5) %	2.20	0.21	-	3.48	8.80	2.42	0.40	-
Lingual tuberculum (type 6)	-	-	-	-	-	-	-	-
Margoid	23	-	-	-	23	-	-	-
(type 8) %	0.67	-	-	-	4.60	-	-	-
No. central cusps	168	19	8	51*	76**	12	2	-

*Simultaneous occurrence of buccal bulging and lingual conical cusp on same tooth in 5 cases
**Simultaneous occurrence of buccal bulging and lingual conical cusp on same tooth in 4 cases

vary according to the population examined (Dankner et al. 1996a).

The separately developed central cusp on the lingual crown side (type 2) was the least frequent (5 premolars and 1 molar). Kutscha (1985) originally described this form on premolars, and Schulze (1987) identified it as the type 2 form of central cusp. In Schulze's classification of central cusps on premolars, this form is close to the type 1/3 form. We did not distinguish between the two forms, and it is described in our material as type 2. The case of a Hungarian girl investigated by Kocsis (1984) involves also the same form.

A new central cusp form (type 8) was also recorded. This phenomenon does not seem to have been reported previously in the literature on central cusps. This trait appeared on premolars, forming an enamel crest and binding the buccal enlargement on the lingual cusp and the lingual bulging on the buccal cusp. It therefore involves a type 1 and a type 5 central cusp form connected to each other via an enamel ridge. The dentin layer also displayed this structure. On the molars, it was seen as an enlargement of the transversal crest between the mesiolingual and distobuccal cusps. From a morphological point of view, this condition is similar to the margoid differentiation on the anterior teeth (de Jonge 1959), and the anomaly may therefore be referred to as a "margoid central cusp formation" (Fig. 9). On all the posterior teeth, its prevalence was 0.64%; it was seen most frequently on mandibular first premolars (4.40%). In the course of the examinations, the simultaneous occurrence of central cusp forms was also recorded. The forms more frequently appeared symmetrically for types 5, 6 and 8, but the other types (1 and 2) more often appeared asymmetrically. The types appeared on various teeth, with the same type (type 5) on premolars and molars on both the upper and lower dentitions. The different types occurred together in one dentition infrequently; type 1 with type 5, type 5 with type 6, or type 5 with type 8. However, an association of different forms on one tooth was found only for bulging of the lingual aspect of the buccal cusps and cone-shaped lingual cusp enlarged in the buccal direction on premolars: this occurred on 18 teeth (10 first and 8 second mandibular premolars) (Tables 3 and 4). This association may be the initial form of type 8 (two central cusp forms without binding).

From the aspect of the associations of the central cusps with other dental abnormalities, it seems that the "margoid central cusp formation" may be connected with compression of the premolar crown in the bucco-lingual direction. Of the 3,970 premolars investigated, 17 mandibular premolar crowns exhibited a comression form, 11 of which were connected with the above-mentioned margoid central cusp.

It was also observed that central cusps figured on the teeth of 2 of the males, among the 17 patients with cleft (5 females and 12 males). Central cusps associated with syndromes were

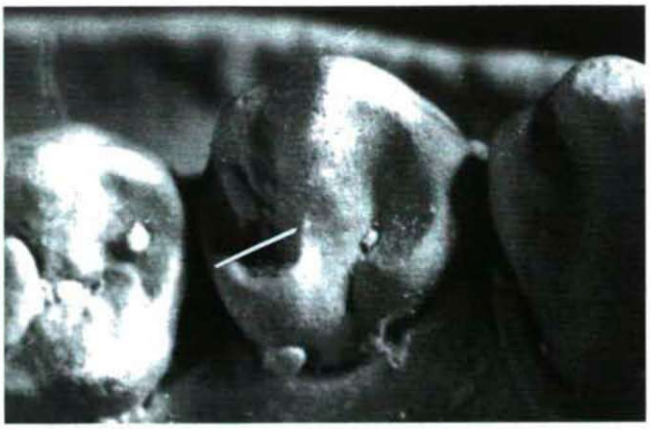

Figure 9. "Margoid central cusp formation" on a lower left first premolar in the investigated material.

not found either on anterior teeth or in the posterior region of the dentitions examined. These syndromes appear very rarely and did not feature in our patients.

From the total of 13,793 teeth, the number with central cusps was 501 (3.69%). (The total number of central cusps was 519, but in 18 cases types 1 and 5 appeared together on the same tooth.) The teeth most affected were the mandibular first premolars (11.6%), second mandibular premolars, maxillary first premolars, and molars also displayed these traits quite often, they were less frequent on the mandibular incisors.

The male teeth to female teeth ratio relating to central cusps was 212:307 (p=0.000015). In both sexes, most of the affected teeth exhibited bulging on the buccal cusp, then cone-shaped lingual cusps and margoid central cusp formation. However, enlarged lingual cusps and separately developed central cusps on the lingual side of the crown were more frequent in males.

The dens evaginatus, a form of central cusp is most frequently discussed in the literature. Population studies of the irregularity and the examination of some families (Merrill 1964; Oka et al. 1964) indicate that the dens evaginatus is inherited autosomal and dominant (Stewart et al. 1978). At the same time, Pearlman and Curzon (1977) regarded it as a developmental aberration. Among the patients investigated, two pairs of identical twins displayed the same of central cusps (types 1, 5 and 6). This suggests a hereditary component in the etiology of the central cusp forms studied.

Conclusion

The "central cusp" is a group term, since the types of central cusps differ, and there are large differences in the frequency, the symmetric occurrence and the infrequent simultaneous appearance of different cusp forms, in one dentition or on one tooth. The traits are connected only through their localization: they occur on the occlusal surface, inside the tip area of the buccal and lingual cusps on the posterior teeth and on the lingual surface of the anterior teeth. The importance of the investigation of the central cusps is in outline as follows.

For dental practice among the central cusps the dens evaginatus and the talon cusp are of significance. The elevation sooner or later becomes damaged and produces a secondary pathology that manifests itself as pulpitis, periodontitis, periostitis, among others.

The dens evaginatus and the talon cusp are the central cusp forms most widely investigated in an effort to learn. The talon cusp has been reported on deciduous teeth, but the deciduous dentition has not yet been investigated with regard to the other cusps. The connection between the talon and some syndromes are well known, and the dens evaginatus on the anterior and on the posterior teeth appears in multiple anomaly forms (*e.g.*, lobodontia), but there has been no description of its appearance in syndromes. Determination of the genetic factors involved in the production of central cusp forms is desirable.

The other traits described in this paper are dental polymorphisms, and their existence enlarge the battery of traits that can be used in descriptions of recent and paleodontological forms of the human dentition. The central cusp forms may be referred to as "non-metric" traits. They occur on all permanent tooth types, and can be used in the examination of population history.

Acknowledgments

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SYMPOSIUM

Anthropological study for the determination of the Europid and Negroid characteristics on facial bones of human fetuses*

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ABSTRACT During my 3-month long scholarship in the Smithsonian Institute in 1991, I carried out metric and comparative anatomical (anthropological) examinations on the collection of fetal/newborn skeletons of the Anthropological Institute of the National Museum of Natural History, Washington, D.C. I determined 85 sizes of 50 characteristic bones of about 350 complete fetal/new-born skeletons in standardized conditions. In the study of facial bones and during the mathematical-statistical analyses, the data of 37 Europid and 27 Negroid and 47 mixed (Mulatto) skeletons were taken into account. According to my analyses the facial bones of the two human races differ significantly in both forms and sizes. The most characteristic formal anthropological features can be identified on the frontal process of the maxilla, the surface of the palate of the maxilla, the plate forming the nasal septum (vomer), the os zygomaticum and the mandibe.The applied mathematical-statistical methods (multiple comparison (Bonferroni), variance analysis, regression and correlation analysis and multivariant discriminant analysis) confirmed the anthropological characteristics and the varied formal differences that are visible to the naked eye. In the forensic medical and the anthropological practice, the possibility of the discrimination of the characteristics of the two main races (and possibility of the determination of the Europid and the Negroid characteristics) can be used with reasonable professional accuracy.
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KEY WORDS

forensic fetal osteology
mathematical-statistical evaluation of the facial bone measurements
Europid and Negrid subracial differences of the human fetal facial bones

The forensic anthropological methodology and the forensic application of the study of fetal bones were laid down in our monograph entitled "Forensic Fetal Osteology" (1978). In this encyclopaedic study we summarized the fundamental information that was available in our own metrical studies and in the literature that may be necessary to take into account during the identification and forensic medical study of human fetal/newborn bones and skeletons of unknown origin in order to acquire the most important theoretical and practical knowledge (Fazekas and Kósa 1978).

In the present study we proposed a model for the determination of body length and actual age on the basis of the sizes of bones for forensic medical and anthropological practice with statistic charts, regressive diagrams and so-called multipliers that determine the body length and with other methods. This practice has become a well-known basic method and it is widely applied.

For a long time the means of proving in forensic anthropology were restricted in the case of fetal and newborn skeletal bones as well as skeletons as compared to the efficiency in the study of adult skeletons (Kósa 1969, 1974, 1978, 1979, 1990a,b, 1993a, 1995a, 1997, 1998a,b, 1999; Kósa and Fazekas 1969, 1972a,b, 1973a,b,c,d,).

Whereas at least 4 individual characteristics can be identified during the forensic anthropological identification of adult skeletons, *i.e.* the actual height of the body, the age, the sex and the main racial characteristics, for a long time it was only an uncertain question in case of fetal/newborn bones. There was no reliable method available on the basis of which the sex and the dominant racial characteristics of fetal/newborn skeletons could have been determined without doubt (Fazekas and Kósa 1965a,b, 1966a,b,c,d, 1967a,b,c, d, 1969, 1978).

In co-operation with my colleagues, using modern statistical methods, we have recently achieved new results regarding the more accurate determination of bone sizes and body height (Huxley 1998; Huxley and Kósa 1999) (Arizona team), the study of fetal sexual dimorphism (Adalian 2001; Adalian et al 2001a,b; Piercecchi-Marti et al 2002) on fetal/newborn skeletons (Marseille team) as well as the study of the morphological characteristics (Casellana and Kósa 1999, 2001a,b,) of the human fetal vertebra which we did not study before (Barcelona team); and regarding the study of racial morphological characteristics and sizes which I carried out on the collection of fetal skeletons in the Anthropological

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*Dedicated to Professor Gyula Farkas on the occasion of his 70th birthday.

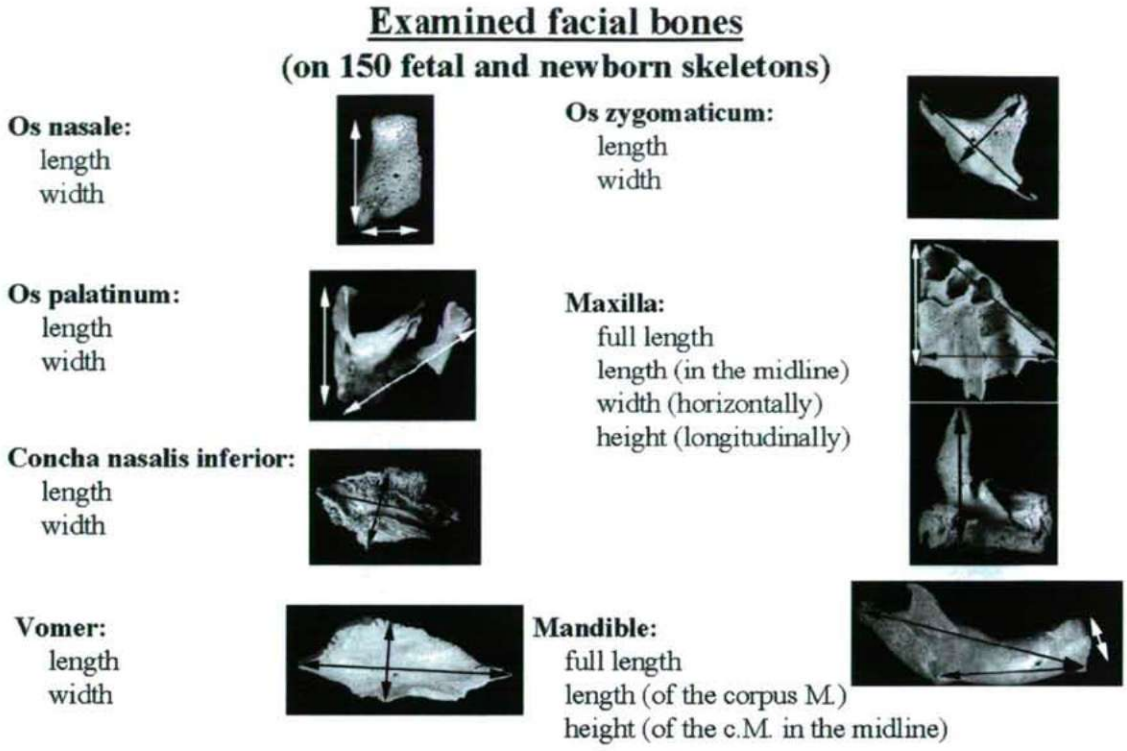


Figure 1.

Institute of the National Museum of Natural History, Washington, D.C. in 1991 (Kósa 1991a,b, 1992, 1993b, 1994a,b, 1995b, 1998a,b, 2000a,b).

In this study I would like to present the morphological racial characteristics of facial bones and the results of statistic evaluations to distinguish between the Europid and Negroid characteristics.

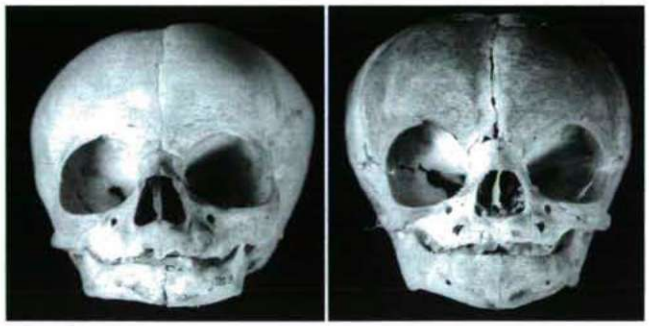
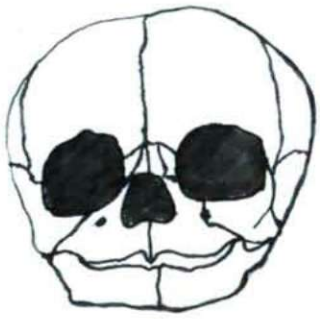


Figure 2.

Figure 3.



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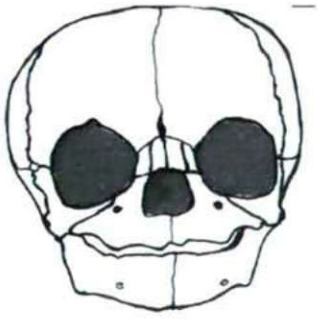
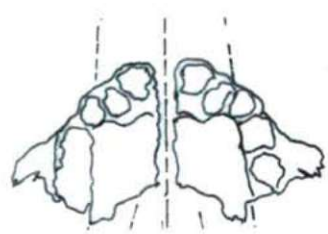


Figure 5.



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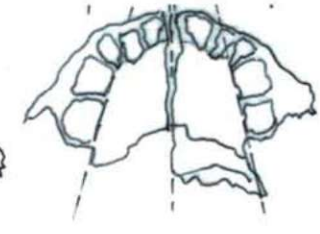

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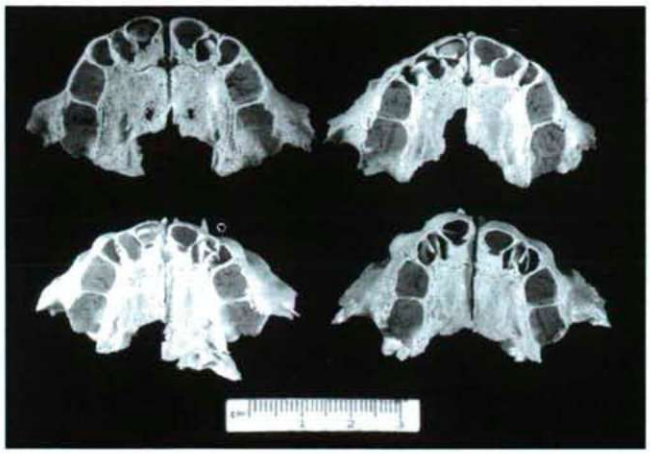

Figure 8.

Materials and Methods

During my 3-month long scholarship in the Smithsonian Institute in 1991, I carried out metric and comparative anatomical (anthropological) examinations on the fetal skeleton collection of the Anthropological Institute of the National Museum of Natural History, Washington, D.C.

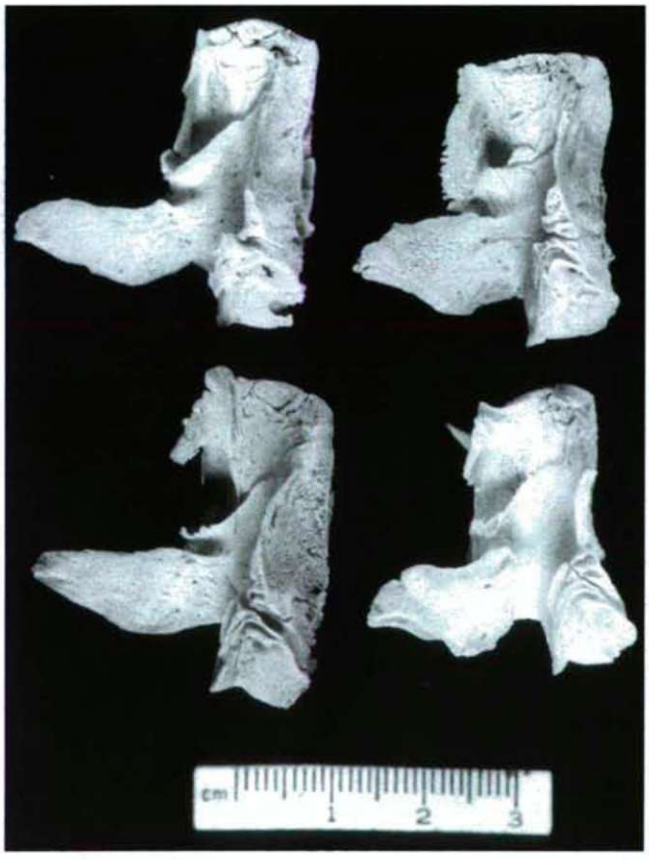

Figure 9.

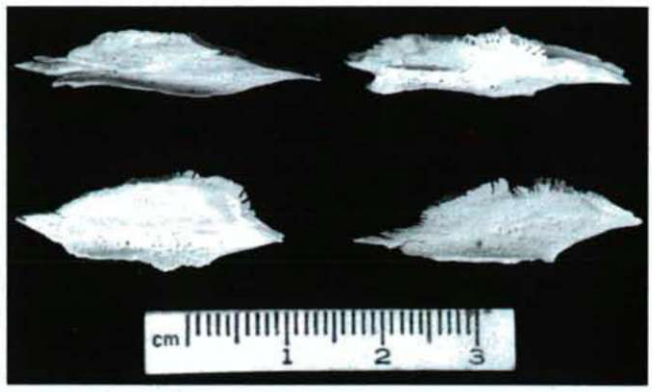

Figure 10.

The importance and curiosity of one of the largest fetal/newborn collection of 350 skeletons (Gindhart 1989) that was collected to be a standard for scientific purposes is that about one-third represent Europid, one-third Negroid and less than a third mixed type (Mulatto) anthropological characteristics. During my studies there I determined 85 sizes of 50 characteristic skeletal bones with standardized measurements. Thus, I acquired 4,250 bone sizes altogether. In recent years I have been publishing data on these bone sizes of different body parts, mainly describing the characteristics of the cranial bones.

In this study I performed the mathematical-statistical analyses of the facial bones on the sizes of 32 Europid, 27 Negroid and 57 mixed (Mulatto) skeletons. The bone sizes were systematically determined by measuring points applied in previous studies (largest bone and width measurements and diameters). In order to express a special anthropological characteristic of a bone metrically, I also used different techniques of measurement.

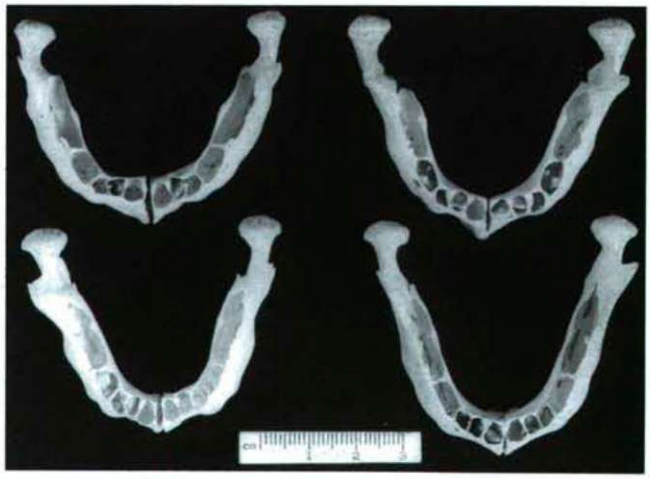

Figure 11.

Multiple comparisons (Bonferroni)

Os zygomaticum (length)	europid-negrid	p<0,037
	negrid-mulatto	p>0,05
	europid-mulatto	p>0,05

Figure 12.

Multiple comparisons (Bonferroni)

Mandible (length of the corpus M.)	europid-negrid	p<0,043
	negrid-mulatto	p<0,074
	europid-mulatto	p>0,05

Figure 14.

In the present study I measured the bones indicated in Figure 1. The arrows indicate the measured distances and the examined bone sizes. During the mathematical-statistical evaluation of the bone sizes the following methods were used: multiplex comparison (Bonferroni), variance analysis, regression and correlation analysis and multivariant discriminant analysis.

Results and Discussion

According to our studies the racial morphological differences within the two races (Europid and Negroid) can be detected as early as in fetal and newborn ages (Kósa 1991a,b, 1992, 1993b, 1994a,b, 1995b, 1998a,b, 2000). The most characteristic racial differences can be detected on the squama of the temporal bone, the frontal process of the maxilla or its palatine surface, the vomer, the lateral plate of the cranial part of the occipital bone and the basal area of the plate of the squama occipitalis.

As stated in our previous publication (Kósa 1996) the intact white and black fetal skulls also show characteristic anthropological differences. These results were achieved on specially prepared newborn skulls in the collection of the NMNH, Washington, D.C., during the preparation of which the soft parts of the skeletons were removed from the bones but the plates and ligaments of interstitial tissues between the sutures that hold together and fixed the calvarial and facial bones in their original position were left intact. Thus, from a didactic point of view, it was managed to prepare newborn skulls that bore the anatomical and anthropological conditions of their original forms.

In order to demonstrate the main racial characteristics, two photographs are shown that were taken in A-P directions. In one of the two photographs (Fig. 2) the skull and the cranial bones of a Europid fetus are illustrated frontally and those of a Negroid one are illustrated frontally in the other (Fig. 3). On the draft about the skulls, the most characteristic anthropological features are indicated (Figs. 4 and 5). While the transversal diameter of the orbitalis aditus is greater than

Multiple comparisons (Bonferroni)

Mandible (full length)	europid-negrid	p<0,001
	negrid-mulatto	p<0,011
	europid-mulatto	p>0,05

Figure 13.

Multiple comparisons (Bonferroni)

Mandible (height of the c.M. in the midline)	europid-negrid	p<0,020
	negrid-mulatto	p<0,030
	europid-mulatto	p>0,05

Figure 15.

Table 1. Multiple comparisons (Bonferroni) of the data.

Dependent variable	(I) R	(J) R	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval Lower bound	Upper bound
VL	White	Black	-2.8720	1.6980	0.282	-7.0126	1.2687
		Colour	-0.6390	1.2417	1.000	-3.6670	2.3889
	Black	White	2.8720	1.6980	0.282	-1.2687	7.0126
		Colour	2.2329	1.5682	0.474	-1.5912	6.0570
	Colour	White	0.6390	1.2417	1.000	-2.3889	3.6670
		Black	-2.2329	1.5682	0.474	-6.570	1.5912
VW	White	Black	-1.1850	0.711	0.320	-2.9959	0.6259
		Colour	0.2977	0.6306	1.000	-1.3081	1.9035
	Black	White	1.1850	0.7111	0.320	-0.6259	2.9959
		Colour	1.4827	0.6737	0.108	-0.2328	3.1982
	Colour	White	-0.2977	0.6306	1.000	-1.9035	1.3081
		Black	-1.4827	0.6737	0.108	-3.1982	0.2328
ZL	White	Black	-4.2844*	1.6805	0.037	-8.3769	-0.1918
		Colour	-1.2916	1.2244	0.882	-4.2734	1.6903
	Black	White	4.2844*	1.6805	0.037	0.1918	8.3769
		Colour	2.9928	1.5622	0.175	-0.8117	6.7974
	Colour	White	1.2916	1.2244	0.882	-1.6903	4.2734
		Black	-2.9928	1.5622	0.175	-6.7974	0.8117
ZW	White	Black	-1.8500	1.2408	0.417	-4.8717	1.1717
		Colour	-3.5880E-02	0.9040	1.000	-2.2375	2.1657
	Black	White	1.8500	1.2408	0.417	-1.1717	4.8717
		Colour	1.8141	1.1534	0.357	-.9949	4.6231
	Colour	White	3.588E-02	0.9040	1.000	-2.1657	2.2375
		Black	-1.8141	1.1534	0.357	-4.6231	0.9949
XF	White	Black	-3.3281	1.5750	0.111	-7.1625	0.5062
		Colour	-1.9397	1.1399	0.276	-4.7148	0.8354
	Black	White	3.3281	1.5750	0.111	-0.5062	7.1625
		Colour	1.3884	1.4582	1.000	-2.1615	4.9383
	Colour	White	1.9397	1.1399	0.276	-0.8354	4.7148
		Black	-1.3884	1.4582	1.000	-4.9383	2.1615
XW	White	Black	-3.1500	1.3129	0.055	-6.3463	4.628E-02
		Colour	-1.6491	0.9502	0.257	-3.9624	0.6642
	Black	White	3.1500	1.3129	0.055	-4.63E-02	6.3463
		Colour	1.5009	1.2155	0.659	-1.4583	4.4601
	Colour	White	1.6491	0.9502	0.257	-0.6642	3.9624
		Black	-1.5009	1.2155	0.659	-4.4601	1.4583
XL	White	Black	-2.0562	1.1616	0.239	-4.8841	0.7716
		Colour	-1.6679	0.8407	0.150	-3.7145	0.3788
	Black	White	2.0562	1.1616	0.239	-0.7716	4.8841
		Colour	0.3884	1.0754	1.000	-2.2297	3.0065
	Colour	White	1.6679	0.8407	0.150	-0.3788	3.7145
		Black	-.3884	1.0754	1.000	-3.0065	2.2297
XH	White	Black	-3.3231	1.7786	0.194	-7.6539	1.0077
		Colour	-1.8103	1.2596	0.461	-4.8773	1.2567
	Black	White	3.3231	1.7786	0.194	-1.0077	7.6539
		Colour	1.5129	1.6525	1.000	-2.5109	5.5366
	Colour	White	1.8103	1.2596	0.461	-1.2567	4.8773
		Black	-1.5129	1.6525	1.000	-5.5366	2.5109
MF	White	Black	-9.3627*	2.4193	0.001	-15.2470	-3.4783
		Colour	-2.9358	2.0278	0.452	-7.8677	1.9961
	Black	White	9.3627*	2.4193	0.001	3.4783	15.2470
		Colour	6.4269*	2.1550	0.011	1.1856	11.6682
	Colour	White	2.9358	2.0278	0.452	-1.9961	7.8677
		Black	-6.4269*	2.1550	0.011	-11.6682	-1.1856
MW	White	Black	-2.5533*	0.9217	0.020	-4.7951	-0.3116
		Colour	-0.4024	0.7725	1.000	-2.2813	1.4765
	Black	White	2.5533*	0.9217	0.020	0.3116	4.7951
		Colour	2.1509*	0.8210	0.030	0.1541	0 .1477
	Colour	White	0.4024	0.7725	1.000	-1.4765	2.2813
		Black	-2.1509*	0.8210	0.030	-4.1477	-0.1541
ML	White	Black	-16.5580*	6.6504	0.043	-32.7331	-0.3829
		Colour	-3.0682	5.5740	1.000	-16.6252	10.4888
	Black	White	16.5580*	6.6504	0.043	0.3829	32.7331
		Colour	13.4898	5.9237	0.074	-0.9177	27.8973
	Colour	White	3.0682	5.5740	1.000	-10.4888	16.6252
		Black	-13.4898	5.9237	0.074	-27.8973	0.9177

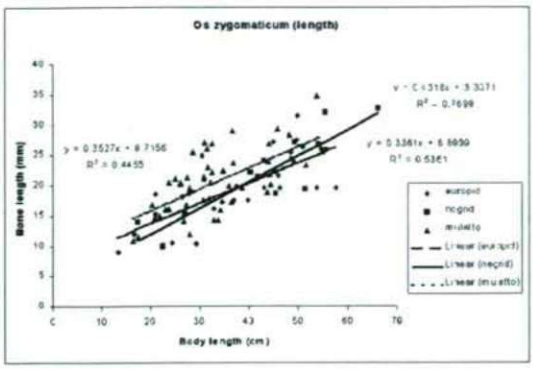

Figure 16.

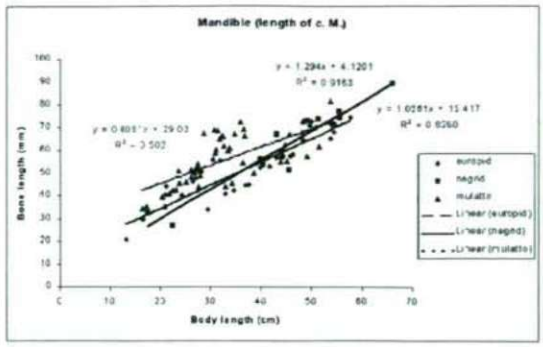

Figure 18.

the longitudinal one in white fetuses, it is just the opposite in black fetuses, namely it is elongated longitudinally. Therefore, the longitudinal size is greater than the transversal size. The bones forming the root of the nose are narrower in white fetuses, therefore, the orbits are nearer to each other. In black fetuses the orbits are wider, therefore, the opening of the nasal cavity is narrower in the upper part and wider in the lower part and is similar to a pear, whereas in black fetuses it has a rounded trapezoid shape. The body and length of the mandible in white fetuses is disproportionately smaller as compared to the cranium cerebrale, whereas it is more massive and more developed in black fetuses.

Little is known about the main racial anthropological characteristics of the intact skull in forensic medical practice. Their fields of application is also limited as the above mentioned morphological characteristics can mainly be recognized by X-ray examinations and not on the dissection table if the cranial bones that have to be studied in order to determine racial characteristics are still tied with ligaments and interstitial tissues.

Certain bones of the skull also show characteristic anthropological differences in the two main races. The palatine surface of the maxilla in white fetuses form a regular semicircular arch. The line of the processus alveolaris is curved backwards semicircularly (Fig. 6), whereas in the Negroid type the initial part of the processus alveolaris of the palatum durum is curved in a semicircle but it follows a line diverging backwards from the canine tooth as far as the 5th grinder (Fig. 7).

The same characteristics can be traced in Figure 8, where the palatine surface of the four facial bones (maxilla) is shown. On the top left part a male fetus, on the top right part a female fetus, on the bottom left part a black male fetus and on the bottom right part a black female fetus can be seen.

The frontal process of the maxilla can be regarded as a characteristic anthropological feature between the two main races as the frontal process of the maxilla is longer and narrower in white fetuses whereas it is shorter and wider in black fetuses. In Figure 9, the anthropological characteristics and morphological differences can be seen on the medial surface of the one side of the facial bone (maxilla) of a male fetus on the top left, that of a white female fetus on the top right, that of a black male fetus on the bottom left and that of a black female fetus on the bottom right. The plate of the nasal septum (vomer) can be seen in the form of longer and narrower bones in Europid fetuses, whereas it is shorter and

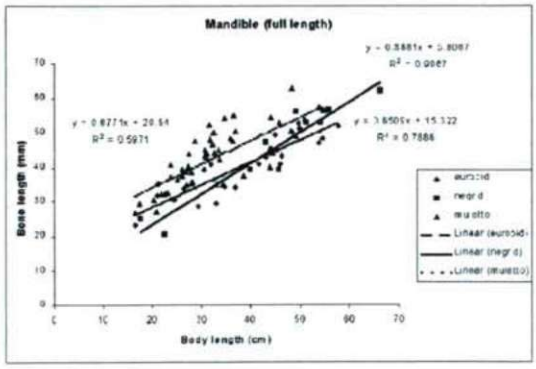

Figure 17.

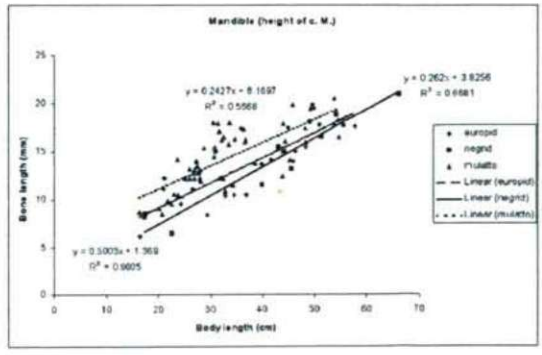

Figure 19.

higher in Negroid fetuses (Fig. 10). The mandible in Negroid fetuses is larger in comparison to the skull as a whole. It is considerably greater both in proportion and size as in white fetuses (Fig. 11).

During the mathematical-statistical analysis of the facial bones we found significant differences in sizes and characteristics not all of the main racial differences but of the easily detectable and visible main racial differences. The transversal size of the os zygomaticum, the full length of the mandible, the length of the body of the mandible, the length of the mandible at medium height both in white-black, white-mulatto and black-mulatto groups showed significant differences. In Table 1, the results of multiple comparisons are indicated. In Figure 12, the significant probability values (P) of the studied size of the os zygomaticum, and in Figures 13, 14 and 15, those of the mandible bone are shown.

Figure 16 shows the regressive lines and equations of the transversal size of the os zygomaticum. Figure 17 shows those of the full length of the mandible, Figure 18 those of the length ot the body of the mandible and Figure 19 those of the height of the mandible measured at medium height together with the regressive coefficients.

It could also be determined in regression analysis that the regression lines and equations differed from one another in the two main races but the regression line of the diagram was in a different position in the Mulatto type as well. However, the correlation coefficients indicated close relationship between the sizes of tested bones and body length. When analysing the linear regression in the three racial human groups (Europid, Negroid, Mulatto) it can be stated that the regression line of the Mulatto is between the Europid and Negroid lines proving a mixed status of the mutual anthropological characteristics.

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SYMPOSIUM

Human adaptation in the 7th-11th century*

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ABSTRACT This paper is an attempt to reconstruct human adaptability in the case of populations which lived in the central region of the Carpathian Basin between the 7th and 11th century. On drawing a parallel between the ecological zonality and the human anatomical patterns of the three historical periods included, we come to a conclusion that the populations of both the Late Avar period (670-894 A.D.) and the time of the Hungarian conquest (10th century, *i.e.* 895-999 A.D.) adapted themselves to the local ecological zonality fairly well, while, from 1000 A.D. on, *i.e.* at the time of the 11th century when the early Christian Hungarian Kingdom was founded by King St. Stephen, it may have been political intention more than anything else that influenced the structure of population. **Acta Biol Szeged 46(1-2):91-94 (2002)**

KEY WORDS

Late Avar period and early Hungarians
multivariate anatomical analysis
historical human ecology

The 7th–11th century human adaptation in the central region of the Carpathian Basin is especially suitable for examinations since this area was reached by numerous ethnic groups in several migration waves. The population of the Late Avar period (670-894 A.D.) was succeeded by the pagan Hungarians, who immigrated and settled down in the 10th century (895-999), then followed the foundation of the early Christian Hungarian Kingdom by King St. Stephen, which marked the beginning of our third period from 1000 on. For the sake of simplicity, we shall refer to these epochs as Late Avar period, 10th century population and 11th century population.

Little is known about the contemporary environmental factors which were of great importance with a view to human adaptation. This is the reason why we lean on recent sources as essential proof. Former experience makes us assume that a reconstruction of the environmental complex in the Carpathian Basin can primarily rely on the specification of climatic zonal regions reckoned by the distribution of precipitation (Borhidi 1961; Varga 1995; Bihari 2000). Over the past centuries the boundaries of the zones have remained nearly the same even though their characters have been varying. This zonal arrangement involves a central plain area surrounded by concentric zones towards the peripheries (Figs. 1 and 2). Thus, the main question is to what extent this zonality is reflected in the anatomical characteristics of the skeletal remains during the three periods mentioned above.

Materials and Methods

Twelve measurements of the skull were examined in each of the three chronological phases. These, marked by Martin (1928) numbers, are as follows: maximum length (M1), basion-nasion length (M5), maximum breadth (M8), minimum frontal breadth (M9), basion-bregma height (M17), auriculo-bregmatic height (M20), bizygomatic breadth (M45), upper facial height (M48), orbital breadth (M51), orbital height (M52), nasal breadth (M54), nasal height (M55). Missing cranial measurement, supposing at least four original measurements were known, were reconstructed by Dear's (1959) principal component method in each of the three periods separately. The samples completed in this way were examined by using the SPSS 7.5 version of the principal component analysis (SPSS 1996) without applying Kaiser's normalization. The human anatomical regional pattern was reconstructed on the basis of the locality averages of the factor values and by employing the 6.02 version of the Surfer programme packet (Figs. 3, 4 and 5).

The craniological data of 883 males from the Late Avar period, those of 321 10th century males and those of 365 11th century males were analysed. The number of localities in the above chronological order was as follows: 67, 60, 22. The reason why male dimensions were only analysed was that in each of the three chronological phases a principal component (a face factor) in which the same original measurements were concentrated (M48, M52 and M55) could only be evinced with males.

Results and Discussion

The fact that a face factor with the same dimensional background could be pointed out in each of the three chronological phases may refer to the continuity of 7th-11th century population history. This factor represented approximately one fifth of the total variance in each of the three samples (*i.e.* 17.5 % in the Late Avar period, 19.8 % in the 10th century and

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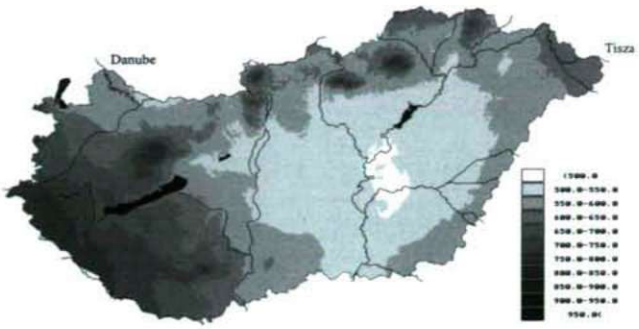

Figure 1. Distribution of precipitation (mm) in Hungary using Aureligh's method (Bihari 2000).

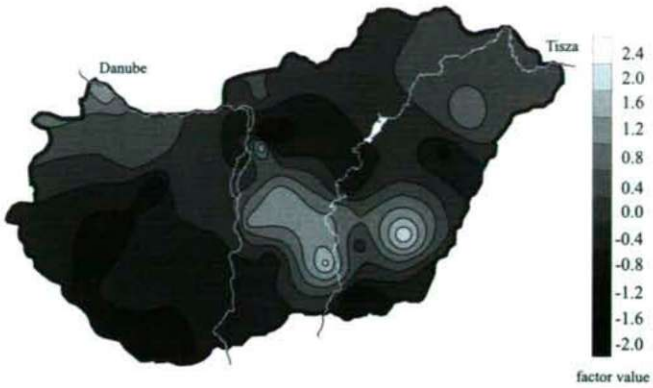

Figure 3. Regional pattern of the face factor of males from the Late Avar period in Hungary (Guba 1999).

18.0 % in the 11th century). Our former analyses had shown that the concentric zonality of ecological zones bore a near resemblance to the regional distribution of the face factor in 10th century populations (Guba and Szathmáry 1999; Szathmáry 2000). That observation, therefore, referred to a well-adapted population. However, we have not yet published our results concerning the populations either in the preceding Late Avar period or in the succeeding 11th century.

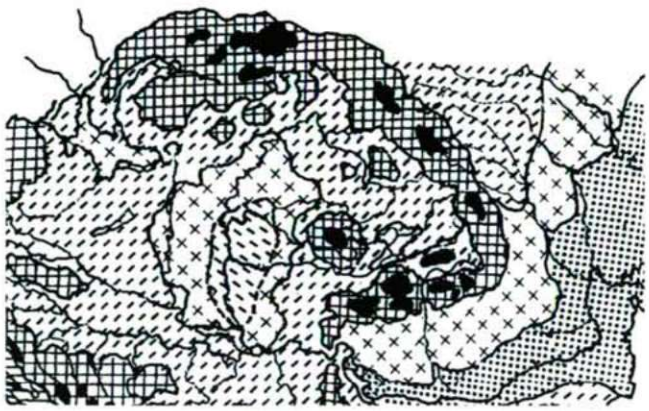

Figure 2. Concentric zonation of bioclimatic regions in the Carpathian Basin (Varga 1995).

We sum up the moments which enable us to assess the population history and the adaptability of 7th-11th century populations in process. In the course of our examinations, the spatial anthropometrical patterns in each of the three periods were estimated in the same respects.

Late Avar period

Conclusions regarding Late Avar populations rely on observations shown in Figure 3.

1) The north-south direction course of the river Danube might have formed a barrier south of Csepel Island. While high-faced people might have lived by far in the greatest number in the east, the western regions might have been inhabited by low-faced populations.

2) North of Csepel Island, however, an anatomical zone of Transdanubian nature characterized by the low face spanned over the river taking a southern direction towards the people of a similar nature living in the northern periphery of the Great Plain.

3) Expanding southwards along the valley of the river Tisza, this low-faced population wedged itself in the masses of the high-faced variants living in the central Great Plain and divided their masses in two.

4) High-faced components appeared exclusively in the middle regions of the Great Plain, both between the two rivers, the Danube and the Tisza, and east of the Tisza. The low-faced group drawing southwards along the river-basin of the Tisza seems to have formed a distinct anatomical boundary zone between the two groups of the high-faced population.

5) The populations which lived in the northwestern and northeastern regions of the territory of present-day Hungary might have been heterogeneous anatomically.

Summing up what was observed, we could say that the anatomical pattern as regards its basic characteristic features bore a resemblance to ecological zonality (cf. Guba 1999).

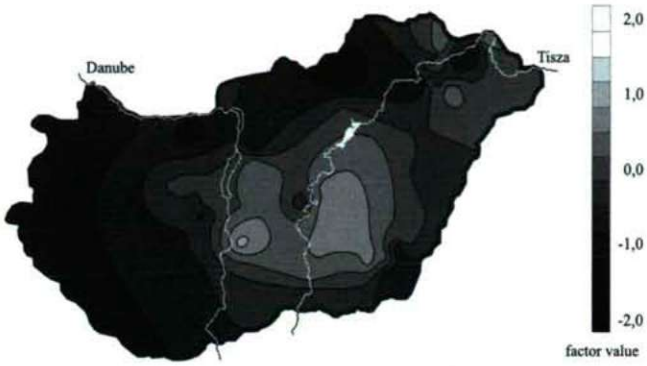

Figure 4. Regional pattern of face factor of 10th century males in Hungary (Guba and Szathmáry 1999).

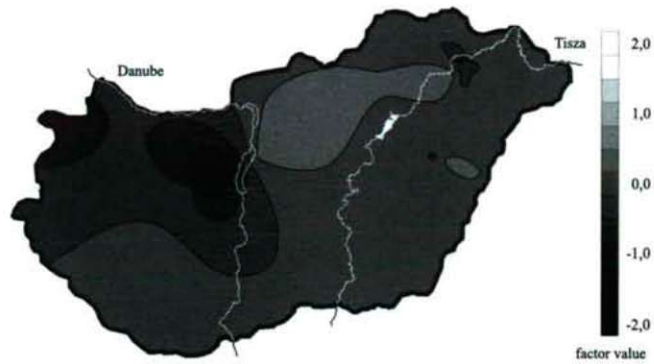

Figure 5. Regional pattern of the face factor of 11th century males in Hungary.

10th century (the age of Hungarian conquest)

On drawing a parallel between the conclusions regarding the Late Avar period and the observations concerning the regional anatomical pattern of 10th century populations given in Figure 4, we can describe 10th century populations as follows (cf. Guba and Szathmáry 1999; 2001):

1) South of Csepel Island, the river Danube continued to form a barrier between the low-faced and high-faced populations even in this period.

2) North of Csepel Island, however, the anatomical zone which had established connections between low-faced people characteristic of the Transdanubian region and the Great Plain populations of similar features narrowed down owing to the expansion of high-faced populations.

3) In the central region of the Great Plain, in the middle reaches of the Tisza, low-faced people lost ground.

4) The two former centres of the high-faced components in the middle region of the Great Plain became merged, therefore the middle reaches of the river Tisza no longer separated populations, on the contrary, brought them into contact.

5) The population in the northeastern region became even more heterogeneous and showed almost all the anatomical variants which could be found in the Carpathian Basin at the time.

To sum it up, we could say that the coincidence of the anthropometrical patterns and ecological zonality looked surprisingly close. Taking this evidence into consideration we could not argue against László's (1970) hypothesis of the so-called "dual conquest". What is more, it seemed that we should lay a great emphasis on the continuity of adaptation from the 7th century up to the end of the 10th century. It is to be noted, however, that the scantiness of 9th century skeletal remains from the Great Plain is a fact of common knowledge. It could be commented on with the following circumstances in mind: the extremely dry climatic conditions (cf. Györffy and Zólyomi 1994) on one hand, and the small number of typically 9th century archaeological finds on the other. The latter makes the majority of archaeologists to date the burials back to the "infallible" 8th century to avoid being exposed to presumable professional criticism.

11th century (early Christian epoch)

Our observations concerning the epoch of early Christianity were as follows (Fig. 5):

1) South of Csepel Island, the river Danube only denoted a lax contingent boundary zone between populations.

2) With a population structure altered in the course of time, a population historical and anatomical zone could be observed north of Csepel Island, along the Danube bend, which, in contrast to the previous periods, separated the populations. Nor this fact neither the insularity of the surviving low-faced population in the surroundings of Székesfehérvár could be explained without referring to civilization intentions.

3-4) The population in the middle region of the Great Plain became definitely homogeneous. No moments from the former population structure could be pointed out. Surprisingly, this observation was in accordance with our earlier results, which showed that, in the 11th century, population history might have been influenced, first of all, by civilization or political factors (Szathmáry et al. 1996; Szathmáry et al. 1997).

5) Even the earlier anatomical heterogeneity in the northeastern and northwestern regions turned into a more homogeneous pattern.

To sum it all up we could state that population structure completely changed in the 11th century. Civilization influences (like urbanization, political intensions, etc.) might have overpowered adaptational potential more adequate to ecological zonality (cf. Szathmáry and Guba 2001).

Acknowledgments

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SYMPOSIUM

Paleopathological changes in the Carpathian Basin in the 10th and 11th centuries*

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ABSTRACT The existence of skeletal materials and accompanying archaeological subsistence data recording the shift from nomadic animal husbandary to sedentary agriculture during the 10th and 11th centuries in the Carpathian Basin offers the opportunity to document shifts in the frequencies in various disease categories. On the basis of the data from the literature of the paleopathological cases, skeletal samples representing 714 tenth and 970 eleventh century individuals are grouped for indications of environmental stress: specific disease stress (porotic hyperostosis), infections, traumatic lesions, degenerative arthritis and genetic and/or environment indicators (developmental anomalies). The frequency of lesions in the samples should not be extrapolated to the larger population, but may only be used as an indicator of a trend in the appearance of the diseases. The results suggest some significant shifts for some disease and little change for other diseases during the transition from a nomadic to a more sedentary way of life.
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KEY WORDS

paleopathology
subsistence patterns
skeletal pathology
Carpathian Basin
10th -11th century

The Hungarian tribes settled down in the Carpathian Basin at the end of the 9th century. The nomadic lifeways of the Hungarians rapidly transformed to sedentary living within the timespan of a few decades. Although the biogeography of the Carpathian Basin, having much in common with the forested steppeland of Eastern Europe, is basically unsuitable for the nomadic life; this homeland offered superb possibilities for intensive stockbreeding and agriculture. Not only did their lifeways change to sedentary agriculture, but their political organization changed and the foundation of the state began. Their pagan beliefs survived until the reign of King St. Stephan (11th century) when they were supplanted by Christianity.

The ethnic groups of the Hungarian Conquest period were heterogenous. They included communities of ancient nomadic pastoralists interspersed with a large number of agriculturists (who had come to the new homeland with a tradition of sedentism from the east as well as the indigenous peoples living in the Carpathian Basin; László 1986; Fodor et al. 1996).

The Hungarian Coquest period and the later centuries have long been investigated according to anthropological and paleopathological aspects. In order to determine whether land use and cultural changes in the 11th century are reflected in the frequency and type of pathological lesions in human remains, the results of paleopathological analysis of human skeletal material dated to the 10th and 11th centuries are presented.

The paleopathological cases have been classified into two groups: 1) diseases indicative of the environment (porotic hyperostosis, infectious diseases, trauma, osteoarthritis; Goodman et al. 1984), and 2) lesions caused by genetic and/or environmental indications (developmental anomalies; Barnes 1994). Some of these lesions might be characteristic of the sedentary changes in life styles between the 10th and 11th century.

Environmental indicators

Specific disease stress: porotic hyperostosis

The adoption of sedentary farming was apparently accompanied by a decline in the overall quality of nutrition. The clearest indicator of this may be provided by the increased incidence of porotic hyperostosis/cribra orbitalia. This is considered indicative of an anemia or nutritional deficiency and its expressive pathological symptom in the bones is porotic hyperostosis. It is manifested as a widening of the spongy diploe with a corresponding thinning of the outer cortical bone table resulting in the appearance of surface porosity. In severe cases there is a total obliteration of the bone surface with a lattice of trabecular overgrowth (Ortner and Putshar 1981; Goodman et al. 1984). These lesions either first appear or show a frequency increase with sedentary farming suggesting that anemia is primarly a disease of these agricultural groups (Goodman et al. 1984).

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Infections

The clearest major trend in the collected data concerns the incidence of infections as measured by the frequency of nonspecific skeletal lesions of infectious etiology as well as by the frequency of certain specific diseases categories.

Infections were a more common and more serious problem for sedentary than for nomadic peoples, and most researchers suggest that this is a result of some combination of increasing sedentary, larger population aggregates and the well established synergism between infection and malnutrition. Infectious diseases are now among the most significant selective factors acting on human populations. Syphilis, tuberculosis, leprosy and osteomyelitis occasionally produce significant bone reactions in advanced stages of the disease. Infections and most other health problems are more commonly found in later farming sites than in either earlier or later nomadic groups (Goodman et al. 1984).

Traumatic lesions

Traumatic lesions have been classified by Steinbock (1976) into four types: fractures, crushing injuries, bone wounds caused by sharp instruments and dislocations. These occur as a result of violent encounters with environmental hazards and inter- and intraspecies conflicts. Of these lesions, fractures and bone wounds by intentional instrument use (trephination) are mainly significant in archeological populations (Manchester 1983). Fractures' rates may also be studied relative to settlement patterns and means of subsistence. As a microtraumatic fracture, spondylolysis was also studied (Merbs 1995), but its etiology is not clear. Trephination is caused by the intentional use of a sharp instrument for the removal of part of the skull vault, generally without damaging the underlying meninges and brain. This dangereous operation was performed for a variety of reasons: to alleviate the intracranial pressure produced by compressed fractures of the skull vault, to clean the wounds caused by fractures, to treat headaches, epilepsy, or other forms of mental illness and so on (Ortner and Putshar 1981). The trephination may also have been symbolic or ritualistic in its type. The symbolic trephination (without opening the cranial cavity) is an operative intervention when a small fragment of the cranial bone is removed from some area of the calvaria after death (Marcsik and Szalai, in manuscript).

Degenerative arthritis

Degenerative arthritis is a common condition in all skeletal populations. Bone changes are most visible around the margins of vertebral body surfaces or either on or peripherial to the margins of the articular surfaces of appendicular joints. These degenerative changes may result from the activities that produce mild chronic, single acute or repeated severe functional stress (Steinbock 1976; Ortner and Putshar 1981).

The most informative approach to the study of degenerative arthritis is to compare frequencies of involvement in different populations, controlling for the demographic variables of sex and age. The frequency of degenerative arthritis is correlated with age (Goodman et al. 1984).

Genetic and/or environmental indicators

Developmental anomalies (malformations)

The alterations due to developmental anomalies may be grouped into several broad classes: disorders of congenital, traumatic, metabolic, inflammatory, and neoplastic origin have been reported in the paleopathology literature. Some anomalies are indentifiable in the newborn, but not all defects are detectable at birth. The majority of such defects remains undetected until exacerbated by growth and development, and appears in childhood or later in adolescence. The range of expression varies, based on a combination of genetic and/or environmental factors which usually interfere with the timing of developmental events. Attempts to categorize these developmental anomalies have been made by a number of authors: "errors" (Zimmerman and Kelley 1982), "failures" (Manchester 1983), or "morphogenetic" (Barnes 1994).

Developmental anomalies are localized on the skull (for example, cleft-formations, premature fusion of the sutures), in the spine (mainly spina bifida occulta, sacral spina bifida, block-vertebra, sacralization, lumbarization), and in the appendicular skeletons (clubfoot deformity, hip dysplasia, etc.). The incidence of developmental anomalies appears to be highest in the spine, lower in the skull and lowest in the appendicular skeleton (Ortner and Putshar 1981; Barnes 1994).

Materials and Methods

The skeletal collections of the Department of Anthropology, University of Szeged, Hungary, served as source material for the paleopathological studies by the different authors. The cemeteries of Szegvár-Oromdűlő (Marcsik 1997), Magyarhomoróg-Kónyadomb (Csányi 2001; Szigeti 2001), Püspökladány-Eperjesvölgy (Pauditz 1995; Finnegan et al. 1997; Spigelman et al. 1999) were chosen as each has components of both the 10th and 11th centuries. The samples involve a total of 374 individual skeletons dated to the 10th century and 970 dated to the 11th century. Since the number of specimens dated to the 10th century was far less than those dated to the 11th century, we also took into the investigation the paleopathological results from two single 10th century samples: Algyő (Marcsik and Szalai, in manuscript) and Sárrétudvari-Hízóföld (Oláh 1990; Pálfi et al. 1996). These paleopathological investigations were carried out *via* gross and radiographic observations of skeletal remains and some cases were analysed for DNA of *Mycobacterium leprae*. The total number of individuals is 1,684, as seen in Table 1.

Results

It is important to emphasize that the frequency of the lesions in our samples should not be extrapolated to the larger population, but may only be used as an indicator of a trend in the appearance of the diseases.

Szegvár-Oromdűlő

Based on this paleopathological investigation of the 10th and 11th century, the main trends of the various diseases and lesions are as follows. Mild types (porotic and cribrotic) of porotic hyperostosis, and its most serious form (trabecular or hyperostosis spongiosa orbitae, cranii), traumatic lesions displayed as fractures and symbolic trephination, sacralization, spina bifida and sacral bifidum as developmental anomalies, and degenerative arthritis were considered both in the 10th and 11th century samples of this cemetery. However, the frequency of each is higher in the 11th century sample. Among infectious diseases, the nonspecific infectious category was unimportant, but osseous tuberculosis was only found in the 11th century sample (Marcsik 1997).

Magyarhomoróg-Kónyadomb

There is a large difference between the 10th and 11th century samples because only a smaller part of the cemetery is from the 10th century and its larger part is from the 11th century. Thus, the occurrences of pathological deformations mainly relate to the 11th century. In a few instances, scaphocephaly, other developmental malformations, osseous (spine) tuberculosis, osteomyelitis (mild type) and the symbolic trephination can be observed while the occurrences of traumatic lesions (fractures), porotic hyperostosis and degenerative joint diseases (osteoarthritis in the hip, spondylitis) are more frequent, and there are no signs of osseous leprosy at all (Csányi 2001; Szigeti 2001).

Püspökladány-Eperjesvölgy

There is a small difference between the 10th and 11th century samples in this material. Mild types of porotic hyperostosis have a higher frequency in the 11th century sample. Of the infectious diseases, osteomyelitis and osseous leprosy were only found in the 10th century sample. *Mycobacterium leprae* DNA was detected in two cases by Spigelman et al. (1999). However, serious skeletal tuberculosis was only seen in the 11th century sample. A higher frequency of fractures was found in the 11th century, although surgical and symbolic trephinations are known from the 10th century. Degenerative arthritis and developmental anomalies were seen in both 10th and 11th century samples with the higher frequencies recorded in the 11th century sample (Pauditz 1995; Finnegan et al. 1997).

Table 1. Distribution of the number of specimens.

Sites and authors	10th	11th	Total
Szegvár-Oromdűlő (Marcsik 1977)	93	259	352
Magyarhomoróg-Kónyadomb (Csányi 2001; Szigeti 2001)	23	345	368
Püspökladány-Eperjesvölgy (Pauditz 1995; Finnegan et al. 1997; Spigelman et al. 1999)	258	366	624
Algyő (Marcsik and Szalai, in manuscript)	77	-	77
Sárrétudvari-Hízóföld (Oláh 1990; Pálfi et al. 1996; Pálfi et al. 1999)	263	-	263
Total	714	970	1,684

Algyő

In this 10th century sample, different types of porotic hyperostosis and the more serious osteomyelitis were seen at relatively high frequencies. There was no sign of any specific infectious disease. Similarly to porotic hyperostosis, higher frequencies of fractures, degenerative arthritis and symbolic trephination were seen. Two special diseases, polyostotic fibrous dysplasia and ankylosing spondylitis were also observed (Marcsik and Szalai, in manuscript).

Sárrétudvari-Hízóföld

The most frequent paleopathological lesions observed in the series can be divided to entesopathies and traumatic lesions (various types of fractures, the signs of chronic strains, surgery and symbolic trephination) and degenerative joint disease (arthritis, spondylosis, spondylarthrosis). The numerical occurrences of bone-joint disorders of non-specific infectious (osteomyelitis, periostitis) and porotic hyperostosis are also relatively high. However, osteonecrosis, osteoporosis, tumors, developmental malformations, spondylarthritis and diffuse idiopathic skeletal hyperostosis can be demonstrated in smaller numbers. It is important to mention that certain signs of deformations caused by osseous leprosy were found in one individual and a most likely occurrence in another one (Pálfi et al. 1996). In these specimens, the results of the molecular biological analysis are negative for the *Mycobacterium leprae* DNA (Pálfi et al. 1999).

Discussion

It seems that the porotic hyperostosis is observed in both 10th and 11th century samples. The more serious types were found during the childhood period in the 10th century sample. Its incidence can vary from site to site in the same general area and time period, and appeared to be dependent on ecological and environmental conditions. A number of etiologies have been suggested as being responsible for the development of these lesions, including anemia or nutritional deficiencies (poor diet), parasitic infections and weaning diarrhea (Stuart-Macadam 1989). Since available protein or other food

nutrients appear to be adequate in the 10th century, it is supposed that 10th century populations might be more susceptible to bacterial infections and/or had reduced iron absorption (due to weaning diarrhea and/or other infections).

Among the nonspecific infectious diseases, cases of chronic osteomyelitis (caused by staphylococcus or streptococcus) are only seen in the 10th century samples. The most serious cases were published by Marcsik and Oláh (1991). Based on the present data, leprosy seems to be more widespread in the 10th century than in the 11th century. There is no evidence of osseous tuberculosis in the 10th century sample: this disease was apparently spread only during the 11th century. It may be supposed that the host-resistance of the Hungarian tribes was reduced against certain bacteria (*Mycobacterium tuberculosis* and/or *bovis*) during the early occupation of this new territory. After the establishment of settlements during the 11th century, when skeletal tuberculosis was widespread, the host-resistance may have been reduced, the tuberculosis bacillus may had become more virulent or there have been a closer relationship (sedentary living) between the vector and the host. The Hungarian plain was well suited for sedentary agriculture and animal husbandry, and its associated human lifestyle could be compatible with the development of tuberculosis. The spread of leprosy in the 10th, of tuberculosis in the 11th, centuries corresponds to the earlier results (Marcsik and Pálfi 1999).

Tuberculosis and leprosy continued to increase in frequency with continued sedentary living, and high frequencies are found in the middle ages in Hungary (Marcsik 1998).

Degenerative arthritis, fractures, and developmental anomalies did not increase significantly during the change from a nomadic to a sedentary life style in the Carpathian Basin. The incidence of both degenerative arthritis and fractures are high in each of the 10th and 11th century samples, but the etiology of each may be different: riding and hunting in the 10th century versus sedentary agriculture and higher population density during the 11th century.

Symbolic trephination in Hungary (apart from four chronologically uncertain cases) can be demonstrated among the populations of the 10th and 11th centuries (Nemeskéri et al. 1960). Even in ethnical respect, the symbolic trephination also relates to the conquering Hungarians of the 10th century. These skulls and their infracranial bones have no other lesions except only one case (grave 242 of Sárrétudvari-Hízóföld with serious osteomyelitis on the femurs and tibias), and there are symbolic trephinations on the infant skulls. The types of symbolic trephination on skulls found in Hungary usually correlate with the symbolic trephination of the 9th and 10th century skulls found in Bulgaria (Boev 1968; Jordanov 1988). Similar interventions are described by Éry (1987, 1988) on skulls found in the Volga region (Tankejevka site, 9th and 10th centuries), while Fóthi at al. (2001) reported 14 skulls with symbolic trephination from the Bolsie Tarhani series (7th-9th centuries).

The examinations of series involving large number of individuals have proved that the practice of symbolic trephination vanished with the consolidation of Christianty in the 12th century (Lipták 1983; Szathmáry 1983). The rate of decline (if there was any decline) in the practice of symbolic trephination (as a pagan trait characteristics) in the 11th century right after the adoptation of Christianity will be answered by further studies regarding the 10th and 11th century samples.

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